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Phytochemical screening and analysis of amino acids of *Magnolia officinalis* seeds

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Abstract
Magnolia officinalis is a woody medicinal plant belonging to the Magnolia family and Chinese traditional medicine. The present study comprises phytochemical screening of different extracts of the seeds of *M. officinalis* and quantitative analysis of amino acids in the seeds of *M. officinalis*. The present study concluded that the seeds contains variety of phytoconstituents. Phytochemical studies on the extracts of seeds showed presence of proteins and amino acid, steroid and terpene, carbohydrates, alkaloids, phenolic and tannins, flavonoids, saponins, fats and oils. This information may be further useful for isolation of various compounds from seeds for treatment of diseases in human beings. Moreover, 17 amino acids were identified in the seeds, of which 7 were essential. The total bound and free amino acids in the seeds of *M. officinalis* was 8065.0 mg/100g and total free amino acids in the seeds was 93.3 mg/100g. The seeds contains abundant amino acids and can be used as potential food additives.

Keywords: seeds of *M. officinalis*, phytochemical screening, metabolites, amino acids

1. Introduction
Plants contained a wide variety of primary constituents and secondary metabolites that are effective in controlling many of diseases. Today, plant materials remain an important resource for combating illnesses, including infectious diseases and many of these plants have been investigated for novel drugs or used as templates for the development of new therapeutic agents, food additives, agrochemicals and industrial chemical [1, 2].

Magnolia officinalis is a woody medicinal plant belonging to the Magnolia family, and has been record in the Chinese Pharmacopeia [3]. This plant is mainly distributed in Asia, such as China and Japan. In China, it is broadly distributed in Sichuan, Hunan, Hubei, Guangxi Provinces [4]. The bark from stems, roots and branches of *M. officinalis* have been used as a folk medicine in the treatment of depression, cough, asthma, liver disease, and diarrhea for more 2000 years [5]. And many reports have been studied that *M. officinalis* has antitumoral, antiangiogenic, antioxidant, and anxiolytic activities *etc.* [6-8]. As the seed of *M. officinalis* is not the medicinal part, a lot of seeds were left unused. The limited studies of *M. officinalis* seeds were concentrated on dormancy, germination, seedling growth and characters [9-11] and phenolics and volatiles in the seeds [12]. The value of plants lies in some chemical substances that produce a definite physiological action on the human body [2]. The phytochemical screening of plant materials with the purpose of discovering new bioactive compounds is an important routine activity before investigating a new plant material. The present study was conducted with an objective to carry out a preliminary phytochemical analysis of the various solvent fractions extracted from *M. officinalis* seeds. Moreover, Quantitative analysis of amino acids in *M. officinalis* seeds was studied in this study. The results are represented in the present study.

2. Materials and methods
2.1 Collection of Seed Samples
The seeds of *M. officinalis* were collected from Pingwu county of Sichuan province, China, in November 2017, some of which were milled in a Sl-200 high speed multi-function pulverizer. The milled seeds were dried in a constant temperature oven (40℃) before use.

2.2 Preparation of tested solution [13, 14]
Water extract: Crude powders of seeds (6.0 g) were added into 80 ml distilled water. After cold leaching for 24 h and filtration, 10 ml filtrate was used as cold water solution to detect the protein and amino acid. The residues were heated in water bath at 70℃ for 45 min. After filtration, the filtrate was used as heated water solution to detect the carbohydrates, saponins. Ethanol extract: 6.0 g crude powders of the seeds were added into 80 ml of 95% ethanol. After reflux extract in water bath for 45 min, filtration was carried out. The residues were added into 50 ml of 95% ethanol and treated with reflux extract in water bath for 45 min again. Filtration was carried out. The all filtrate was concentrated into 60 ml. Then, the concentrated sample was divided into 2 groups: 30 ml of sample, so as to prepare ethanol solution, was detect the phenolic compounds and tannins, flavonoids, cardiac glycosides, quinones, steroids and terpenes. The rest 30 ml of sample was concentrated into extract and then added into 30 ml of 1% hydrochloric acid. The part dissolved in hydrochloric acid was used to detect the alkaloid. Petroleum ether extract: 6.0 g crude powders of the seeds were added into 60 ml petroleum ether. After reflux extract in water bath (60℃), filtration was carried out. The filtrates were concentrated for the detection of fats and oils.

2.3 Phytochemical Evaluation [1, 2, 13-17]
The freshly prepared tested solutions were qualitatively

analyzed for the presence of major phytochemical constituents using following standard procedure.

Detection of Alkaloids

a) Mayer's test

To the 1 ml of ethanol extract add Mayer's reagent (1-2 drops) in a test tube. This leads to formation of a creamy pale yellow or white precipitate indicates the presence of alkaloids.

b) Silicotungstic acid test

To the 1 ml of ethanol extract in a test tube, Silicotungstic acid (1-2 drops) was added. This leads to formation of a pale yellow or off white precipitate indicates the presence of alkaloids.

Detection of phenolic compounds and tannins

a) Ferric chloride test

To take 1 ml of ethanol extract in a test tube and add 1-2 drops of 1% ferric chloride solution. Formation of bluish-green to black-green color indicates the presence of phenolic compounds and tannins.

b) Ferric chloride - potassium ferricyanide test

1 ml of ethanol extract was taken and dropped on the filter paper. After drying, ferric chloride-potassium ferricyanide reagent was sprayed. Appearance of blue spot indicates the presence of phenolic compounds and tannins.

c) Vanillin - hydrochloric acid test

Ethanol extract was taken and dropped on filter paper, and then sprayed with vanillin - hydrochloric acid reagent after drying. Formation of varying degrees reddish of spots indicates the presence of phenolic compounds.

Detection of flavonoids

a) Aluminium trichloride test

To take 1 ml of ethanol extract in a test tube and add 1-2 drops of 1% aluminium trichloride solution. Formation of yellow color with fluorescence indicates the presence of flavonoids.

b) Shinoda test

Take 1 ml of ethanol extract in a test tube, and a little magnesium powders were added into the test tube and mixed up. And several drops of concentrated hydrochloric acid were added, and the color could be developed within 1-2 min (when necessary, slightly hot).

c) Lead acetate test

To the 1 ml of ethanol extract in a test tube, 2 to 3 drops of 1% lead acetate solution were added. Appearance of yellowish to reddish precipitate indicates the presence of flavonoids.

d) Ammonia-fumigation test

Take the ethanol extract and drop it on the filter paper. After drying, put it on the concentrated ammonia bottle and fumigate for half a minute. The filter paper was examined under ultraviolet light, and the spots were yellow with fluorescence, indicating the presence of flavonoids.

Detection of Saponins

a) Foam test

Take 1-2 ml of hot water extract, put it in the test tube and seal. And it will be shaken violently for 2 minutes. If a large amount of continuous foam is produced, and it is put for more than 10 minutes or heat without obvious reduction, there may be saponin.

b) Trichloroacetic acid test

The hot water extract was dropped on the filter paper, spraying 25% trichloroacetic acid ethanol solution. Saponins can show red spots by heating.

c) Phosphorus molybdic acid test

The hot water extract was dropped on the filter paper, dried. After spraying 5% phosphorus molybdic acid ethanol solution, it can show dark blue when heated to the temperature of 140℃ for 5-10 minutes, which indicates the presence of saponins.

Detection of Proteins and Amino acid

a) Ninhydrin Test

Take 1 ml of water extract and add 2-3 drops of Ninhydrin reagent in a test tube, mix up, boil for about 5 min. After cool down, formation of deep blue color indicates the presence of protein and amino acids.

b) Biuret test

Take 1 ml cold water extract in a test tube, add 10% sodium hydroxide solution 2 drops, shake well, then add 0.5% copper sulfate solution 1 or 2 drops, shake well again. The formation of red color, red-purple color or purple color, which indicates the presence of proteins.

Detection of steroid and terpenes

a) Liebermann–Burchard test

Take 2 ml of ethanol extract in small dish and dry on the water bath. The residue was dissolved with 1 ml glacial acetic acid, then added 1 ml acetic anhydride and 1 drop of concentrated sulfuric acid. If the reaction solution color displayed successively yellow - red - purple - blue - deep green, it indicates the presence of steroid. If reaction solution only displayed successively yellow - red - purple, it indicates that there may be triterpene compounds.

b) Chloroform - sulfuric acid test

Take 2 ml of ethanol extract in a dish and dry on the water bath. The residue was dissolved with 1 ml chloroform, and transferred to a small test tube, added 1 ml of sulfuric acid along the side of the test tube. Chloroform layer showed red or blue and green fluorescence was observed in sulfate layer under UV lamp, which indicates that there may be steroidal compounds.

Detection of fats and oils

a) Oily spot test

One drop of the petroleum ether extract was placed on the filter paper and then the solvent was allowed to evaporate. Appearance of oily stain on the filter paper indicates the presence of fixed oil.

b) Phosphomolybdic acid test

Take the petroleum ether extract on the filter and dry, spraying 25% phosphomolybdic acid ethanol solution and heating for 2 minutes (115-118℃). Spots were blue under the yellow-green or navy background, which indicates that the fats and oils.

Detection cardiac glycosides

a) Kedde test

Take 1 ml of ethanol extract and add 3, 5-dinitrobenzoic acid reagent (3-4 drops). The formation of red or purple indicates the presence of cardiac glycosides.

b) Legal test

Take 1 ml of ethanol extract in a dish and put it on the water bath to dry. Residues was dissolved with 1 ml pyridine, and added 4-5 drops of 0.3% nitrous acid sodium cyanide solution of iron, mixed up, added 10% sodium hydroxide 1 to 2 drops, mixed up. reaction solution was red and then color faded gradually again, which indicates the presence of cardiac glycoside.

Detection of carbohydrates

a) Fehling's test

Take the 1 ml of hot water extract in a test tube and add 4-5 drops of Fehling's reagent, heat for 5 minutes in a water bath. Formation of red precipitate indicates the presence of reducing sugar.

b) Molisch's test

Take 1 ml of heated water extract in a test tube and add 2-3 drops of Molisch's reagent, add 1 ml concentrated sulphuric acid carefully along the side of the test tube. Formation of violet color at the interface of two liquids indicates the presence of carbohydrate.

Detection of Quinones

a) Borntrager Test

Take 1 ml of ethanol extract and add 1 ml of 10% sodium hydroxide solution. If the reaction solution was red, 5 drops of 30% hydrogen peroxide were added, heated. If the red color didn't fade, several drops of 5% hydrochloric acid were added. The red color was gone, which indicates the presence of quinones.

b) Magnesium acetate test

Take 1 ml of ethanol extract and add 1 to 2 drops of magnesium acetate methanol solution (1%). The formation of red indicates the presence of quinones.

c) Boric acid test

Ethanol extract was taken and dropped on the filter paper. After drying, 1% boric acid solution was sprayed. The appearance orange yellow or red spots with fluorescence observed under ultraviolet lamp indicates the presence of quinone.

2.4 quantitative analysis of amino acids

Quantitative analyses of free and bound amino acids in the samples were investigated on a HITICHI L-8800 automatic amino acid analyzer manufactured in Japan with a separation column, a reaction column and an automated injection apparatus, which was used the post-ninhydrin method to determine the various amino acids in the sample. The following equipment and conditions were used: A sample analysis cycle 53 min; Separation column (60 mm × 4.6 mm): an eluent flow of 0.4 ml/min, column temperature 70℃, column pressure 9.627 MPa; Reaction column: ninhydrin and triketone buffer flow of 0.35 ml/min, column temperature 135℃, column pressure 1.078 MPa.

The method of extracting free amino acids from the seeds of *M. officinalis* was refer to T. V. Ilina [18]. Free amino acids were determined by placing raw materials (1 g, accurate weight) into a plastic centrifuge tube (5 ml), adding sulfosalicylic acid solution (2 ml, 6%), extracting for 30 min by ultrasound, placing in refrigerator overnight, and treating with ultrasound for 30 min again. The treated solution was centrifuged at 22000 rpm/min for 3 min and the supernatant was diluted 2 times by using sulfosalicylic acid solution (2 ml, 6%). The resulting supernatant was filtered through 0.22 μm filter membrane and analyzed.

The method of extracting total free and bound amino acids from the seeds of *M. officinalis* was refer to L. L. Garmaeva [19]. Total free and bound amino acids were determined by weighing raw materials (0.12 g) in a test tube (150 mm × 15 mm) and adding HCl solution (10 ml, 6 N), mixing. The test tube was hermetically sealed after 10 min of vacuuming and stored at 110℃ for 24 h in constant temperature oven for hydrolyzing. When the hydrolysis was finished, the tube was removed from the oven and cooled to room temperature. The hydrolysates were stirred thoroughly and filtered through filter paper, discarding the first portions and collecting the filtrate in containers. The filtrate (1 ml) was placed into a beaker (50 ml), evaporated to dryness on thermostat water bath (60℃), diluted 2 times by adding HCl (0.02 M). The resulting solution was filtered through 0.22 μm filter membrane and analyzed.

3. Result and Discussion

Qualitative phytochemical screening of the seeds of *M. officinalis* in various organic solution extract were analyzed (table1). The present work is helpful in analyzing the quality and purity of the crude drug. From these result, proteins and amino acid, steroid and terpene, carbohydrates were significantly present in seeds of *M officinalis*, and alkaloids, phenolic and tannins, flavonoids, saponins, fats and oils are present in the seeds of *M. officinalis.* However, cardiac glycosides and quinones are not observed in the seeds. Alkaloids, tannins, flavonoids and phenolic compounds are important bioactive compounds in plants [20]. Phenolic compounds and flavonoids have significant antioxidant activity [21, 22]. The seeds of *M. officinalis* can be used as medicinal plant to cure some common ailments and various diseases. The seeds of *M. officinalis* also contain significant proteins and amino acids, which can be potentially used as food additives. The quantitative analysis of the seeds and purity of crude extract will be further studied.

Table 1: Phyto-chemical investigation of seed of *M. officinalis*

Tests	Water extract	Ethanol extract	Petroleum ether extract
Alkaloids	nd	+	nd
Phenolic and Tannins	nd	+	nd
Flavonoids	nd	+	nd
Saponins	+	nd	nd
Proteins and Amino Acid	++	nd	nd
Steroid and Terpene	nd	++	nd
Fats and Oils	nd	nd	+
Cardiac Glycosides	nd	—	nd
Carbohydrates	++	nd	nd
Quinones	nd	—	nd

"++" strong presence, "+" presence, "-" absence.

The protein content in the seeds of *M. officinalis*. was determined by Coomassie Brilliant Blue G-250 method based

on formation of a colored complex resulting from reaction of the protein with coomassie brilliant blue G-250 dye and detected at 595nm by ultraviolet spectrophotometer [23]. The protein contents was 0.45% in the seeds. As a result, 17 amino acids were identified in the seeds of *M. officinalis*, including 17 protein genic acids, of which 7 were essential and 10 were non-essential. The content of bound amino acids was determined by subtracting the content of free amino acids from the total content. The results were showed in Table 2.

The total bound and free amino acids in the seeds of *M. officinalis* was 8065.0 mg/100g and made up 8.07% in the raw material, of which 2.79% were essential amino acids. Total free amino acids made up 0.09% in the raw material, of which 0.02% were essential; and total bound amino acids in the raw material made up 7.97%, of which 2.77% were essential. Essential amino acids were dominated by leucine (21.28%), phenylalanine (17.48%) and valine (17.38%); non-essential amino acids, glutamine (25.18%) and aspartic acid (18.60%). Free essential amino acids were dominated by threonine (66.17%); free non-essential ones, glutamine (48.63 %). The total amino acids were dominated by glutamine (1328 mg/100g).

The amino-acid composition of the seeds of *M. officinalis* was studied for the first time.

Table 2: Amino-acid Composition of seeds of *Magnolia officinalis* (mg per 100 g of Raw Material)

Amino acid	Bound	Free	∑free and bound
Essential amino acids			
Thr	373.7	13.3	387.0
Met	36.6	0.4	37.0
Val	484.1	0.9	485.0
lys	407.1	0.9	408.0
Phe	486.4	1.6	488.0
leu	593.9	0.1	594.0
Ile	389.1	2.9	392.0
∑	2770.9	20.1	2791.0
Non-essential amino acids			
Asp	975.6	5.4	981.0
Ser	448.4	2.6	451.0
Gln	1292.4	35.6	1328.0
Gly	459.2	0.8	460.0
Ala	429.6	16.4	446.0
Cys	100.3	0.7	101.0
Tyr	216.8	0.2	217.0
His*	244.7	0.3	245.0
Arg*	413.1	10.0	423.0
Pro	620.8	1.2	622.0
∑	5200.8	73.2	5274.0
Total	7971.7	93.3	8065.0

*Conditionally essential amino acids.

4. Conclusion

Phytochemical screening, biological screening of randomly collected plants and their phytochemical examination have proved to be helpful in discovering the new drugs. The present study concluded that the seeds contains variety of phytoconstituents. Phytochemical studies on the extracts of seeds showed presence of proteins and amino acid, steroid and terpene, carbohydrates, alkaloids, phenolic and tannins, flavonoids, saponins, fats and oils. This information may be further useful for isolation of various compounds from seeds for treatment of diseases in human beings. Moreover, 17 amino acids were identified in the seeds, of which 7 were essential and 10 were non-essential. The total bound and free amino acids in the seeds of *M. officinalis* was 8065.0 mg/100g and total free amino acids in the seeds was 93.3 mg/100g. The seeds contains abundant amino acids and can be used as potential food additives.

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