

Research Article

Determination for Synthesis and Content of Tetrahydropalmatine Based on High Performance Liquid Chromatography

Yuanming Zhang

Laboratory of New Fiber Materials and Modern Textile, The Growing Base for State Key Laboratory, Qingdao University, Qingdao 266071, P.R. China

Abstract: Tetrahydropalmatine is a kind of food additive with useful medicine value (dietary supplement), the tetrahydropalmatine synthetic process by using high performance liquid chromatography method was researched in the study, the experiments show that the dissolution rate of active ingredients in the tetrahydropalmatine water extract synthesized by this method has increased and the amount of active ingredient has greatly improved.

Keywords: High performance liquid chromatography, synthesis, tetrahydropalmatine

INTRODUCTION

Coptids Rhizoma was extracted from *coptis chinensis* and was the similar alkaloids with similar structure of isoquinoline ring. When the 2, 3, 9 and 10 were replaced by hydroxyl, methylene two oxygen radicals and methoxy, respectively, the corresponding was berberine, jatrorrhizine, coptisine and palmatine. These compounds have the activity of antibacterial, antitumor, antiarrhythmic, hypoglycemic, protect the gastric mucosa and neural regulation (Tomori *et al.*, 1992) (Fig. 1).

The Tetrahydropalmatine was synthesized from Coptids Rhizoma and accomplish the conversion of alkaloids by by three-step reactions: demethylation or demethylene-dioxy, hydrogenation and methylation and the yield was 18%. The structures were confirmed by ¹H-NMR and ESI-MS. The study provides reasonable ideas for the similar structure of alkaloids and has a better guid to improve the medicinal value of Chinese medicine and new drugs research. Tetrahydropalmatine is a central inhibitor and has good application for analgesia, sedation and stability (Wang *et al.*, 2008). It has been widely used in the treatment of traditional chinese medicine and the relieve of addiction symptoms of drug users and addicts and has low toxicity, larger safety. But the lack of resources, shortage of supply and expensive price limit the further application. Tsutomu (Xu *et al.*, 2013) take 3, 4-Dimethoxytoluene as raw material to obtain Tetrahydropalmatine through bromination, acylation. It required fewer steps, but the reaction temperature is -78°C which is difficult to done. Its material includes Lithium aluminum hydride which is expensive and easy to burn. Cutter reported Tetrahydropalmatine is synthesized from Veratraldehyde, but it needs higher conditions, complicated operation, expensive reagent and is not

suitable for batch production. So one of the purposes of the present study is to have the most simple experiment steps to obtain the highest income (Fig. 2).

Tetrahydropalmatine (THP) is an alkaloid found in several different plant species, mainly in the *Corydalis* genus, but also in other plants such as *Stephania rotunda*. These plants have traditional uses in Chinese herbal medicine. The pharmaceutical industry has synthetically produced the more potent enantiomer Levo-Tetrahydropalmatine (Levo-THP), which has been marketed worldwide under different brand names as an alternative to anxiolytic and sedative drugs of the benzodiazepine group and analgesics such as opiates. It is also sold as a dietary supplement (Wang *et al.*, 2008).

Tetrahydropalmatine has been demonstrated to possess analgesic effects and may be beneficial in the treatment of heart disease and liver damage. It is a blocker of voltage-activated L-type calcium channel active potassium channels. It is a potent muscle relaxant (citation needed), it has also shown potential in the treatment of drug addiction to both cocaine and opiates and preliminary human studies have shown promising results (Gao *et al.*, 2009).

Animal experiments have shown that the sedative effect of THP results from blocking dopaminergic neurons in the brain (Du *et al.*, 2012). Dopamine is an important neurotransmitter in the central nervous system where it occurs in several important signaling systems that regulate muscular activity and attention, as well as feelings of joy, enthusiasm and creativity (Xing *et al.*, 1997). Therefore, THP causes no feelings of euphoria and has been seen as an alternative to addictive drugs for people suffering from anxiety and pain and as a possibility for relief for people not helped by existing drugs (Ferrer and Barceló, 1999).

The contents of tetrahydropalmatine, protopine and berberine hydrochloride in the *corydalis* tuber were

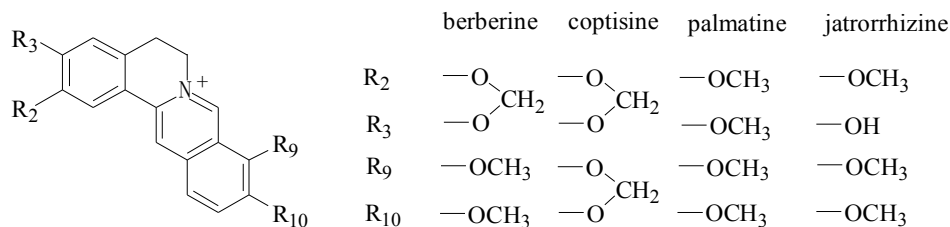


Fig. 1: The structure of alkaloids of rhizome coptidis

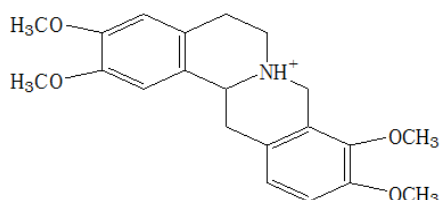


Fig. 2: The structure of tetrahydropalmatine

determined when the high performance liquid is the same in the experiment (Caslavská and Thormann, 2011). At the same time, the titration method was used to conduct the determination for total alkaloid, Corydalis synthesis process was evaluated with multi-index, the latest synthetic methods was applied to synthesize (Portmann *et al.*, 2010). Through the comparison with the traditional processing technology, it strives to find a convenient and efficient synthesis process (Hong *et al.*, 2008).

MATERIALS AND METHODS

Experimental equipment: All the reference substances, such as Agilent-1100 HPLC (DAD detector, autosampler, online degasser, quaternary pump), Galam Galanz microwave oven, PHS-3C precision pH meter, KQ-300 VDB CNC ultrasonic cleaner, BS210S electronic balance, Tetrahydropalmatine, Protopine and berberine hydrochloride were purchased from National Institute for the Control of Pharmaceutical and Biological Products; And Corydalis medicines were purchased from Kangmei pharmaceutical; The acetonitrile was chromatographically pure and other reagents were analytically pure, the water was pure water (Oliferova *et al.*, 2005).

Sample preparation: Preparation of corydalis processed products:

- **Fumarate tablets:** Take the original herbs with uniform size, remove the impurities, wash them clearly and moisture them thoroughly, then cut into slices with certain thickness, keep constant temperature at 50°C for drying.
- **Vinegar-sunburn corydalis:** Take 500 g corydalis with uniform size, mix them with 100 g rice vinegar, conduct covered moistening for 2 h until

vinegar is absorbed, place it in the frying container, heat the mixture with slow fire, after that, remove and let the mixture cool for preparation (Huang *et al.*, 2004).

- **Boil corydalis with vinegar:** Take 500 g corydalis with uniform size, mix them with 100 g rice vinegar as well as proper amount of water, conduct covered moistening for 2 h, heat the mixture with slow fire, boil it until the mixture is cooked through. When vinegar is absorbed, remove and cut them into thick slices, keep constant temperature at 50°C for drying and set aside.
- **Bake the corydalis:** Take 500 g Corydalis with uniform size, add 100 g rice vinegar and stir the mixture, conduct covered moistening for 2 h until the vinegar is absorbed, place the mixture in the thermostatic oven which the temperature is set at 80°C for drying, remove and let the mixture cool for preparation.
- **Sunburn the corydalis with wine:** Take 500 g corydalis with uniform size, add 100 g rice wine and stir the mixture, conduct covered moistening for 2 h until vinegar is absorbed, place the mixture in the frying container and heat them with flow fire for thorough drying, remove and let the mixture cool for preparation.
- **Microwave processing method:** Take 100 g corydalis with uniform size, add 100 g rice vinegar and stir the mixture, conduct covered moistening for 2 h until the vinegar is absorbed, use 60% power of the microwave for heating, which lasts 5 min, remove and let the mixture cool for preparation.

Experiment methods:

Determination for the content of HPLC chromatographic column: Dikma kromasil C18 (250×4.6 mm, 5 μm); mobile phase: acetonitrile-0.2% glacial acetic acid (adjust the pH of triethylamine to 6.0), gradient elution conditions: 0~50 min (15~50% B), 50~70 min (50~80% B), 70~75 min (80% B); flow rate: 1.0 mL/min; column temperature: 30°C; detection wavelength: 280 nm.

Preparation of alcohol extract: Take about 0.5 g sample powder (three screens), conduct accurate weight for the sample and place it in the flat-bottomed flask, precisely add concentrated ammonia solution as well.

Fifty mL methanol (1:20) mixed solution, conduct the weight for the methanol; after quenching for 1 h, conduct heating reflux for 1 h and let it cool, then once again conduct the weight of the methanol solution, at last, use concentrated ammonia solution for mixing. Make up the weight loss of with the same methanol (1:20) mixed solution, shake it evenly and conduct filtration. Precisely take 25 mL of the filtrate, evaporate it and dissolve the residue in methanol, then transfer the solution into a 5 mL volumetric flask and dilute it to the mark, shake it and conduct filtration (0.45 µm membrane), take or add the filtrate, then the target extract is obtained.

RESULTS AND DISCUSSION

The generation of standard curve: Precisely draw the mixed reference solution 1, 2, 4, 6, 8 and 10 µL, respectively pour it into the liquid chromatograph to measure peak area. The result of protopine standard curve: $Y = 8.2874x - 1.3325$ ($R^2 = 0.9997$); the standard curve of berberine hydrochloride is $Y = 1.2874x - 0.7915$ ($R^2 = 0.9995$); the standard curve of tetrahydropalmatine as follows: $Y = 6.1913x - 3.0412$ ($R^2 = 0.9998$); the linear ranges of the three kinds of references are: 0.1408~1.408 g; 0.0416~0.416 g; 0.2408~2.408 g.

Experimental study for precision: Precisely draw 10 µL reference solution of protopine, berberine and tetrahydropalmatine, replicate injections five times according to the above chromatographic conditions, measure the peak area values, the RSD of peak area of protopine, berberine and tetrahydropalmatine are ($n = 5$), 1.19, 2.2 and 1.32%, respectively.

Determination for content: Precisely draw 5 mL corydalis sample solution and infuse it into a dry beaker, evaporate it by low temperature water bath, cool it at room temperature, precisely add 20 mL of 0.02 mol/L sulfuric acid titration solution into it, shake and make it fully dissolved, insert the pen pH meter, use 0.02 mol/L sodium hydroxide for titration. The pH 5.1 is the titration endpoint.

Calculation method: The content of total alkaloid is calculated with Tetrahydropalmatine, each millimeter of sulfuric acid titration with amount-of-substance concentration of 0.02 mol/L is equivalent to 7.1084 mg Tetrahydropalmatine ($C_{21}H_{25}NO_4$). Experimental results show that the content of the total alkaloids in the raw products is 5.48 mg/g, the content of processed products is between 5.09~5.33 mg/g, the microwave processed products have the highest level of content, compared with the raw materials, the content of total alkaloids has reduced to different extent after the corydalis is processed.

CONCLUSION

Experimental results show that, after the corydalis is processed, the contents of protopine, berberine and tetrahydropalmatine in the alcohol extract have reduced to different extents, however, the contents of the above three alkaloids in the water extract have increased to different extents, it may be attributed to the insolubility of drifting alkaloid in water, which can generate soluble salts when using vinegar to sunburn alkaloids so as to improve the dissolution rate and enhance the efficacy, confirming the scientific value of sun-burning corydalis with vinegar; the wine has the ability of desorption, which can promote biological alkali elution from the plant cells, thereby enhancing efficacy. The alcohol extract is prepared by mixing concentrated ammonia solution and methanol (1:20) mixed solution, the alkaloid exists in the dissociation form, which is soluble in methanol and other organic solvents, leading to easier extraction effect than by using water extraction method, but compared with the raw materials, during processing, the active ingredient may be affected by heating, causing decomposition so as to decrease the content.

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