DETERMINATION OF ORGANOPHOSPHORUS PESTICIDES IN HONEYSUCKLE USING MICROWAVE-ASSISTED EXTRACTION

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Abstract A GC-FPD method is described in this paper for the simultaneous determination of 14 organophosphorus pesticides (including dichlorvos, methamidophos, mevinphos, acephate, phorate, disulfooton, orozone, domethoate, diazinon, chlorpyrifos, malathion, parathion, phenthoate, profenofos) in honeysuckle (*Lonicera japonica* Thunb) by using microwave-assisted extraction (MAE) and solid-phase extraction (SPE) cleanup. The effects of different microwave powers, extraction time and solvents on the recoveries have been determined. The main procedure involved that 2g honeysuckle sample ,control or fortified with the pesticides at 0.1-0.5 mg/kg, was extracted by 20ml acetone under 90 W microwave radiation for 5 min.. The extract was mixed with 30ml 3% aqueous Na_2SO_4 solution and then extracted with dichlomethane. The dichlomethane extract was concentrated to near dryness, and resolved in acetone, followed by cleanup with a 500mg C_{18} SPE column. About 3.6 ml elution was concentrated to 2 ml for GC-FPD determination.

The preliminary result under the 90 W microwave radiation for 5 min. showed there were good recoveries for 12 in 14 fortified organophosphorous pesticides, the range from 91.3% to 103.0% with a relative standard deviation (*RSD*) of 2.2%-9.9%. Both methamidophos and acephate have poor recoveries of 19.4% and 35.6%, respectively. The reason was in the step of mixing extract with 3% aqueous Na_2SO_4 solution. The two pesticides could be recovered with recovery of no less than 90% if the aforementioned partition step was not used. But the clean effect was not very good.

Under the microwave radiation power of 150w for 3 min, the recovery would be as good as

that of 90w for 5 min., with the exception of diazinon (less than 80%).

Compared from acetone, to use either ethyl acetate or dichloromethane could obtain the recoveries with higher than 80%, which were not as good as those with acetone.

With comparison of the conventional extraction method, the effect of MAE for 3-5 minutes was equal to or even higher than the conventional method. The recovery of diazinon by conventional method lasting 3 hours was 81.6 %, yet that of 90 w MAE for 5 minutes was 91.3 %. With a high sample throughput, MAE could enhance the extraction efficiency by 35-60 times and during the procedure no decomposition of any of the 14 organo-phosphorous pesticides was obtained.

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Key Words: Microwave-assisted extraction, Solid-phase extraction, Organo-phosphorus pesticides, Honeysuckle *(Lonicera japonica)*

MICROWAVE-ASSISTED EXTRACTION (MAE) OF AZADIRACHTIN-RELATED LIMONOIDS (AZRL) FROM THE NEEM PLANT

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Abstract The neem tree is a tropic plant that is famous for its pesticidal properties (Schumtter, 1990). Study revealed that its pesticidal properties were mainly due to a class of compounds, namely, limonoids which are abundant in various parts of neem tree, especially in the seed (Schmutter and Singh, 1995). The isolation of these limonoids can be carried out by simply soaking the neem sample into a solvent. But the problem is that it is a time consuming process. For commercial neem-based pesticides production, a more efficient technique is required. Microwave-assisted extraction (MAE) is a technique characterized by fast extraction, solvent saving, and high recovery (Pare and Belanger, 1994). Furthermore, this technique is especially efficient for fresh plant samples. Therefore, it is possible to solve the problem by applying the MAE technique in the extraction process.

In this paper we investigated the influence of various factors on MAE of AZRL from various

parts of neem. The AZRL content in the extract was estimated with a colorimetric method and multivariate-calibration technique (Dai et al., 1999). A mathematical modeling method was also used to aid in the spectrum analysis and calculation. The effect of microwave power and microwave irradiation time on the recovery of AZRL was studied for both leaf and seed sample. The efficiency of MAE of seed kernel, seed shell, leaf, leaf stem was compared to the extraction methods RFX and RTE. The results revealed the existence of a special MAE acceleration effect with MAE technique, especially for the leaf and leaf stem samples. The results also suggest that besides the microwave acceleration effect, temperature played an important role in the acceleration. The investigation of the influence of solvent on the MAE of AZRL from seed kernel, seed shell, and leaf samples suggested that the solvent used for MAE can not only affect the extraction efficiency but also affect the component of the extracts.

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METHOD DEVELOPMENT FOR THE MEASUREMENT OF PAH, PASH AND NITRO-PAH IN DIESEL PARTICULATE USING MICROWAVE-ASSISTED SOLVENT EXTRACTION

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A cooperative project among several Canadian government laboratories has been undertaken to study: a) the amount of atmospheric particle in populated areas contributed by transportation sources and b) the impact of transportation fuels and types of vehicles on atmospheric particle loading. In support of these objectives, particles and gases emitted from vehicles must be fully characterized. As part of the research team, the laboratories at ETC have developed an analytical method for the determination of polycyclic aromatic hydrocarbons (PAH), polycyclic aromatic sulfur heterocycles (PASH) and nitro-PAH in diesel particulate.

Microwave energy was used to assist in the solvent extraction of organics from particles as an alternative to the conventional Soxhlet solvent extraction and pressurized solvent extraction (ASE) methods because of cost effectiveness and less environmental impact. NIST reference material SRM 1650 was used to assess the variability, recovery and accuracy of the method. Methodology, validation and levels of target compounds in diesel particulate will be presented.

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MICROWAVE-ASSISTED EXTRACTION OF EFFECTIVE COMPONENTS FROM A CHINESE HERB - CHEQIANCAO

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ABSTRACT

Some influences on the Microwave Assisted Process(MAP), such as solution type and dose, the extraction time, the microwave power etc are studied in this paper by using the Cheqie grass. Compared with the conventional methods, the MAP is characterized by the following advantages: quick process, low energy consumption, high efficiency and less pollution.

USING MICROWAVE PROCESS FOR EXTRACTION OF GINKGO FLAVONE GLYCOSIDES

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ABSTRACT

Laboratory tests were performed to investigate the use of microwave process for the extraction of flavone glycoside compounds from the Ginkgo leaves. A series of extract solvent are preliminarily selected and compared to determine which solvent is more efficient in the process, and the result shows the microwave process is available and cost effective for the extraction of flavone glycosides.

INTRODUCTION

The extract of Ginkgo biloba leaves comprising flavone glycosides, ginkgolides, bilobalid and proanthocyanidins may be one of the most important natural herb medicine in the world. The

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Ginkgo biloba extract has been shown to be effective in treating some synthetic diseases such as cerebrovasular insufficiency, senility, impotence, inner ear dysfunction, premenstrual syndrome, retinopathy, vascular fragility etc. Interestingly, the total extract is more active than single isolated components with removing the accompanying harmful Gingkolic acids (1-3).

The microwave assisted extraction process is a solvent extraction technique whereby target compounds are quickly extracted from plant materials by using microwave energy to a mixture of the treated materials and low dielectric constant solvents (4,5). This process is more effective than conventional solvent extraction technique. The target compounds in conventional extraction is normally enhanced by heating and mixing of the material/solvent slurry. The microwave-assisted extraction uses solvent that is transported to microwave and heating which occurs in localized area within the materials, and forces the target compounds to be released from the materials. Then the target is quickly transferred to the solvent with creating pockets of high temperature and pressures as the material/solvent slurry is exposed to microwaves. As well, the microwave energy level is low and the energy usage is very efficient because the energy is directed exactly to where it is most effect and the percentage of dielectric material with the slurry is not high.

In this work, the test parameters such as solvent type, solvent-to-material ratio, microwave effect time and energy input have been selected and analysed such that they are assessed the application of microwave process to enhance the extraction of flavone glycosides compounds from Ginkgo biloba leaves on the large scale.

MATERIALS AND METHODS

Materials. The dry Ginkgo biloba leaves with a flavone glycoside contend of about 1.5% were purchased from market in Shandong province of China and stored at -20°C in laboratory. The standard reagent of ginkgo flavone glycosides was purchased from Biology Reagent Institute of China, and organic solvents were analytic purity.

Extraction. The tests were performed using a Panasonic microwave oven unit (Panasonic Electri. Inc., Japan) in which a magnetron transmits microwave through waveguide at a frequency of 2450 MHz and at a power of 90 to 900 W. The tests used a glass cylindrical extraction vessel with a diameter of approximately 40mm and a volume of 250ml. The extraction vessel was placed directly in the microwave waveguide located and exposured about 2 minutes. Gingko flavone compounds were extracted three times from the Gingko biloba leaves that are crushed in a mill to a particle size of approximately 4mm. And then it used the extraction solvents (solid/liquid is about 1:15) such as 50% methanol, ethanol, acetone, ethyl acetate, aether, n-hexane at under 80 °C. The extract was filtered, washed with water and concentrated under reduced pressure, and the residue was dissolved in 40 % aqueous ethanol.

Analysis. The final extracted solution was analyzed by high performance liquid chromatography (HPLC) according to references (6,7). The HPLC has carried out on a Shimadzu LC-10AD equipment with a reversed phase column (C18, 250x4.6 mm packed column with a guard column, particle size 5μm), and the column was equilibrated with a mobile phase of MeOH-H2O-COOH (85:15:0.8), flow rate was 1 ml/min. The elute was monitored at 330 nm, 0.2 AUFS, using a scanning UV variable wavelength detector. Identification of flavone glycosides was spiked the retention times under the same conditions with the corresponding standards.

RESULTS AND DISCUSSION

The results of extraction effects corresponding to the different microwave powers of 0, 270, 630, 900 W and the solvents of methanol, ethanol, acetone, ethyl acetate, aether, n-hexane and ethanol/hexane for the open extraction tests were shown in Table 1.The extraction efficiency at 270 W was increased about 23% to 39% (average 31%) than the control group (0 W), in which the samples were placed in a electric oven for 2 minutes at under boiling point of the solvents. As shown in Fig.1, 2, when the microwave power was been increased from 270 W to 630W and 900 W, the change of extraction efficiency was different according to the varied solvents. For n-hexane and ethanol/hexane, the extraction effect has a few increases

Table 1. Effect of different solvents and microwave powers on the flavone glycosides extraction.

No.	Solvent	Microwave power(W)				Relative Content (weight%)			
		1	2	3	4	1	2	3	4
1	methanol	0	270	630	900	9.5	13.2	13.4	13.5
2	ethanol	0	270	630	900	10.3	14.1	14.2	14.4
3	acetone	0	270	630	900	10.2	14.2	14.4	14.3
4	ethyl acetate	0	270	630	900	9.4	13.5	13.7	13.8
5	aether	0	270	630	900	10.7	13.3	13.5	13.4
6	n-hexane	0	270	630	900	8.5	11.1	11.5	11.8
7	Ethanol/hexane	0	270	630	900	10.4	14.5	14.8	15.1

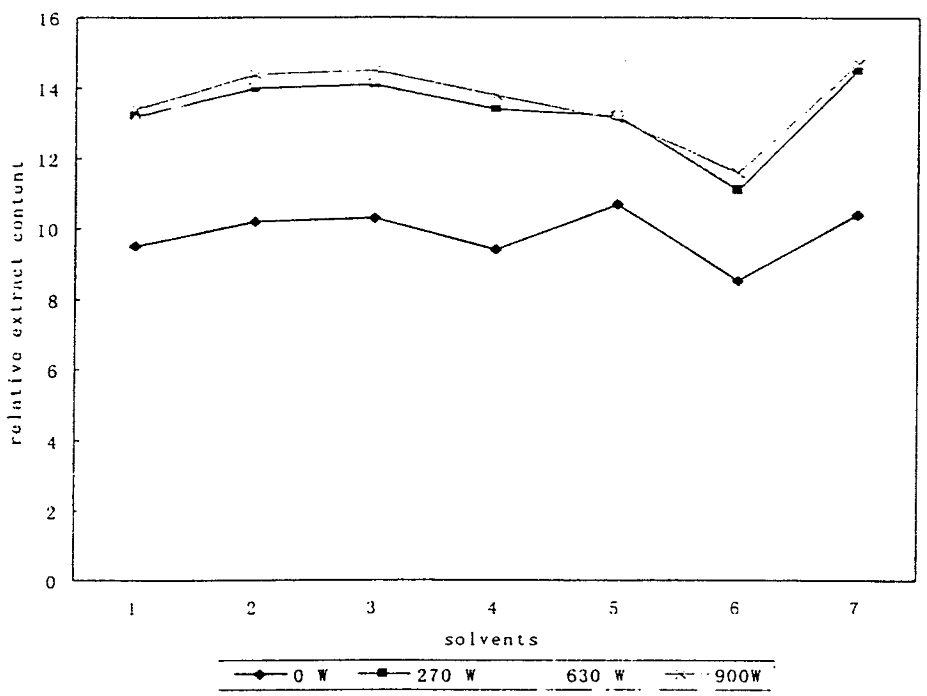
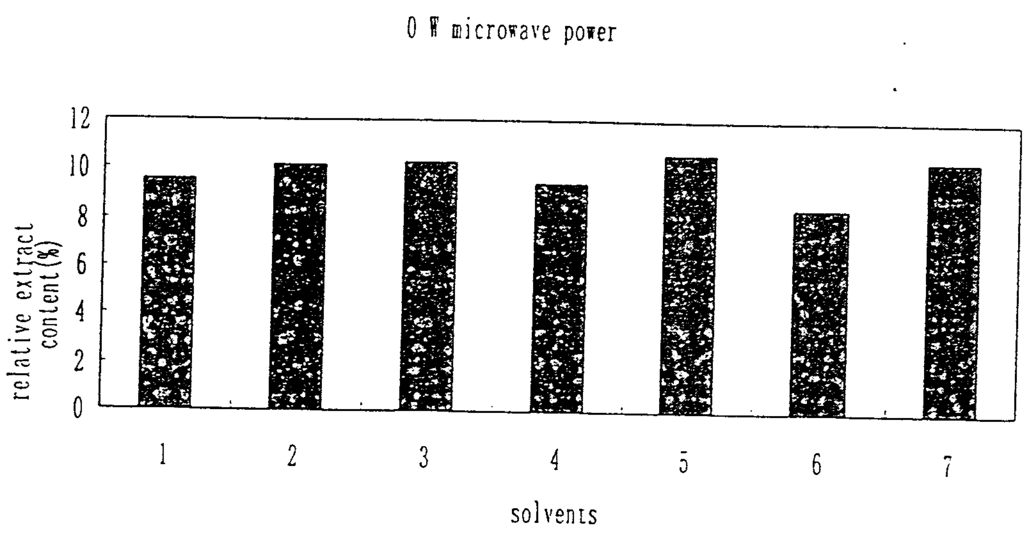
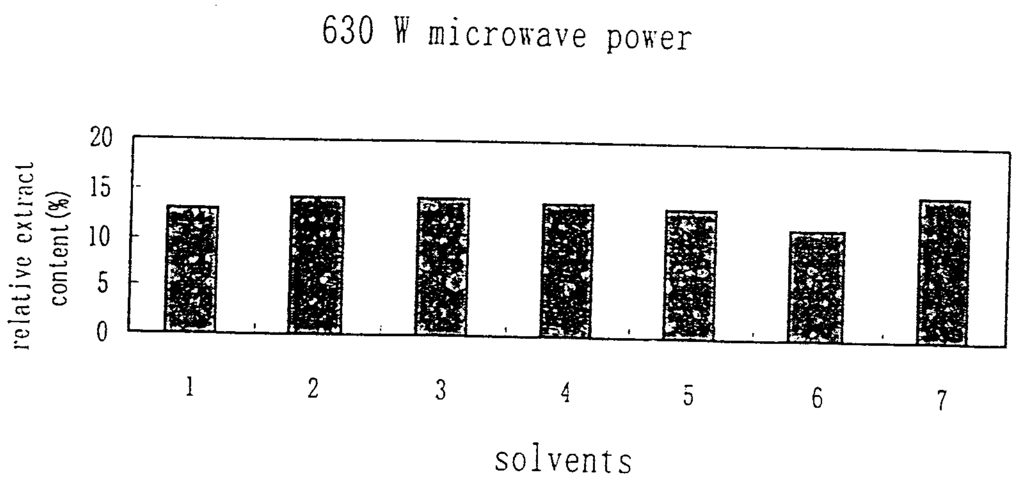


Figure 1, Extraction efficiency corresponding to microwave powers of 0W, 270W, 630W, and 900W



A, 0 W power



B, 630W power

Figure 2, Extraction effect of exposure in non-microwave and microwave condition.

(About 2% to 6%), but the extraction efficiency was no difference (within the statistical) for methanol, ethanol, acetone, ethyl acetate and aether in the tests. Because the non-polarity of hexane is stronger than that of the other solvents in the test, and the microwave is relatively easier absorbed by the polar molecules. So the hexane is easier solubilize the non-polar and higher molecular weight compounds in the leave than the methanol, ethanol, acetone methanol, ethanol, acetone etc., and it would be needed more microwave energy to effect the target compounds in cell of the plant leave. This results would suggested that, at greater than a basic microwave power of extraction effect (may be 270 W in this test) that was put into the extraction process did not contribute to any enhancement for some relatively polar (hydrophilic) solvents in the extraction mechanism.

As also be seen in Table 1and figure 2, for the control group (0W), the range of extraction effect of the solvents was aether >ethanol/hexane > ethanol >acetone> methanol >ethyl acetate > n-hexane. For the microwave effect groups, the effect range of the solvents was ethanol/hexane > acetone > ethanol > ethyl acetate > aether >methanol > n-hexane. Because the boiling point for aether or methanol is much lower than ethanol/hexane and hexane, the rotary evaporation step and microwave process was shorter for the aether or methanol than for the other five solvents, so the extraction effect of the aether or methanol was lower than that of other solvent. There was no significant difference effect of extraction between the acetone and ethanol in these tests, and the ethanol/hexane is the first effect extraction solvent. Of course, the acetone or ethanol may be the first choused solvent for the commercial reason, and the further investigation must be preformed to determine the best microwave process.

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THE RESEARCH AND APPLIED PROSPECT OF MICROWAVE EXTRACTING THE EFFECTIVE CONSTITUENTS FROM CHINESE HERBAL MEDICINE

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Abstracts: Traditional Chinese medicine is taken by the medical juice after the herbal medicine had been cooked with water and filtered the herbs. The method is not only tedious formalities, takes more time but also no taking the medicine in time and carrying it in journey. In order to be convenient to take the medicine, the modern Chinese herbal medicine is processed into the tablet, pill, liquid or injecting medicine. These processed Chinese herbal medicine is easy to be taken and carried, taken care of and quality period is longer. The routine methods to process the Chinese herbal medicine are that the herbs with water or alcohol are cooked, then the cooked herbs removed and the medical juice is concentrated, the effective constituents are separated and refined from the concentrate, at last, the special additives are added to the effective constituents and we will get the processed Chinese medicine. The former methods leaching the Chinese medicine is bad selective, higher impurity, difficult for separating and refining, longer leaching time, and more expensive solvents and energy. Researchers are now looking for a new and valid leaching method to Chinese medicine. The Chinese medicine gotten by the new method will be more effective and less side-effect. Microwave extraction is a good technique and method which has higher selective, quickly, higher recovery, less expensive solvents and energy for leaching material from different plants. Generally, the effective constituents of Chinese herbal medicine are the compounds with hydroxyl, carbonyl, carboxyl, pyridyl or amino group. The vicine or convicine extracted from the fava beans and gossypol extracted from the cottonseed by microwave heating shows the yields obtained by microwave method are 1.3 to 3 times of that obtained by traditional method. But the time used by microwave method is one eighth of the traditional method. The study on microwave-heated leaching saponin from the Paris polyphylla Smith var. yunnanensis (Franch.) Hand Mazz and sallidroside, tyrosol from Rhodioda sachalinensis A. Bor. shows that the yields gotten by microwave heating are higher than that gotten by traditional heating method, but the time exhausted by microwave method is only one twelfth of the traditional method. Microwave is a radiation which is different to -ray. The microwave does not destroy the molecular structures of the material extracted from plants but the -ray does. It shows that microwave radiation will not cause the damage to the effective species extracted from the herbs and not produce the harmful material. The advantages of microwave-assisted extraction will provide a new applied prospect to produce the new Chinese medicine for us. We are engaged in the research of microwave-assisted extracting the effective constituents of Chinese herbal medicine and will introduce the new development in future. This paper will introduce some researching result of microwave-assisted leaching the effective constituents from Chinese herbal medicine.

MICROWAVE-ASSISTED EXTRACTION OF GLYCYRRHIZIC ACID FROM LICORICE ROOT-EFFECT OF THE PROPERTY OF SOLUTION ON PERCENTAGE EXTRACTION OF GA*

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Microwave digestion, using aqueous mineral acid, of matrices for their eventual elemental analysis has been routinely used for several years. [1] Recently, Some applications of MAE for biologically active compounds have appeared in the literatures. [2,3] Licorice, the roots of *Glycyrrhizia glaubra*, is well known for its clinical application to the treatment of stomach ulcers. Now, it is used extensively in the tobacco, food, confectionery, and pharmaceutical industry throughout the world. Glycyrrhizic acid is the main active constituent in licorice root. Microwave-assisted extraction of glycyrrhizic acid from licorice root was more effective than the conventional extraction methods due to the considerable saving in time and solvent [4].

The property of solution is an important factor influencing the extraction efficiency. In the present work, the effect of the property of solution on percentage extraction of GA was studied, which including the concentration of ethanol, ammonia and cation, pH of solution, different organic solvent etc. The results show that 50-60%(v/v) ethanol can reach high percentage extraction of GA. If 1% (v/v) ammonia solution was added into 60%(v/v) ethanol, the percentage extraction can be increased from 2.0% to 2.31%. Without ammonia, 50mmol/L [M+] (M+=Li+, Na+, K+, NH4+ etc.) was added into 60% ethanol, percentage extraction of GA can reach about 2.10-2.26%. If more than 100mmol/L [M+] was added, the percentage extraction of GA decreased. If solution pH was adjust to pH=4 or 13, it can reach high percentage extraction. Solution pH was adjust to pH<3.0, the percentage extraction decreased because glycyrrhizic acid participated partly. If solution pH (60% ethanol + 50mmol [M+], pH=6.10) was adjust to pH=4.0, especially M+ is K+ or NH4+, it can reach almost same extraction efficiency as that of 1% ammonia solution + 60% ethanol, and the operation environment can be greatly improved. As for solvent, ethanol, methyl alcohol and acetone achieved almost same results at same experimental conditions.

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MICROWAVE-ASSISTED EXTRACTION: A NOVEL METHOD FOR EXTRACTION OF BIOLOGICALLY ACTIVE COMPONENTS FROM CHINESE HERBAL MEDICINE*

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China is endowed with abundant resources of medicinal plants. Traditional Chinese medicine represents an accumulation of long period practical experience in treating human diseases. During the past few decades, research and development of these traditional medicines has attracted serious international scientific attention. A great number of these medicinal plants have been more or less investigated chemically in many countries. In general, extraction of biologically active components from Chinese herbal medicine is a weak link, it needs several hours, even more time, but the extraction efficiency is very low. So how to transfer biologically active components high efficiently, fast, selectively and economically from Chinese Herbal medicines to solution is one of important, but arduous task.

More recently, microwave-assisted extraction (MAE) has been investigated because it (1) reduces extraction time (2) reduces solvent and energy exhaust (3) improves extraction efficiency and selectivity. It has been used to extraction of various types of components from soil, seeds, foods, feeds, plant, etc. In our research work, a household microwave oven was modified with the addition of a magnetic stirrer, water condenser, temperature measurement and time controlling. The application of microwave-assisted extraction of biologically active components from Chinese herbal medicine has shown great potentials comparing with conventional extraction techniques. The advantages are discussed, illustrated by recent developments, including:

Microwave-assisted extraction of glycyrrhizic acid from licorice root with ethanol, ethanol-water, water-ammonia or ethanol-water-ammonia.

Microwave-assisted extraction of polar compounds (protocatechuic aldehyde and protocatechuic aicd) with water and non-polar compounds (cryptotanshinone, tanshinone-IIA and tanshinone-I) with ethanol or other organic solvent from *Salvia miltiorrhize Bunge.*

The results show that using the optimal condition, the yields of the extracted compounds obtained by MAE was equal to, even more than that of obtained by the conventional extraction techniques, such as extraction in room temperature (ERT), the traditional Soxhlet, heat reflux and Ultrosonic extraction. But the MAE procedure employed reduces solvent, is quicker at least 5-10 folds and is less labor intensive. MAE can enhance the extraction of biologically active components from medicinal plant. This novel method is suit for fast extraction of Chinese herbal medicine.

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