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Extraction of bioactive chemical compounds from the medicinal Asian plants by microwave irradiation

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The microwave-assisted extraction (MAE) system is an efficient technique under the optimal condition in the extraction of bioactive chemical compounds E- and Z- guggulsterone, cinnamaldehyde and tannin from the plants has been investigated. The results show that, this method provides a fast and easy procedure for the extraction compared to the conventional extraction techniques.

Key words: Microwave-assisted extraction, plant, E- and Z- guggulsterone, tannin, cinnamaldehyde.

INTRODUCTION

In recent years, the electromagnetic energy in the range of microwave has gained special attention in regard to various fields of utilization such as domestic, analytical and one of biomedical applications. Many organic reactions assisted by microwave heating have the advantages of enhanced selectivity and much improved reaction rate. Other advantages of microwave-assisted reactions are milder reaction condition and formation of cleaner products with higher yields, minor wastes, environmental compatibility and minor energy consumption. Organic chemistry has achieved extensive improvements from microwave irradiation with the help of domestic oven, which was the first to be used in the organic reaction, mainly using the radiation of 2450 MHz frequency. Recently, commercial ovens have been designed for kilogram scale preparative reactions. More recently, mono-mode reactors, which here allowed conducting different reactions- by their size of using a maximum of reactants (30 - 40 g), are the best system able to allow the measurements and control of temperature throughout the reaction which proceeds with a good homogeneity and high energetic yield (Loupy et al., 1998).

The microwave-assisted hydro distillation (MAHD) (Lucchesi et al., 2004; Lucchesi et al., 2006) is a new technique for the production of essential oils, solvent-free microwave-extraction (SFME) (Lucchesi et al., 2004). A combination of microwave-heating and dry distillation is also a new green technique developed in recent years. Hot extraction filtration (HEF) method, developed by MLS GmbH is also a new equipment and suitable for the extraction of plants (Ondruschka and Asghari, 2006). The influence of microwave and CO₂-super critical fluid extraction (SFE) on the extraction of medicinal plant in the presence moisture was reported (Sonnenschein et al., 2002). In the case of extraction, numerous applications have been reported on the use of microwave for assisting extraction from plant materials but only a few papers exist (Sha et al., 2004).

Microwave irradiations are absorbed by ions in solution, solvent and dipolar compounds. Since glass and much polymeric material are nearly transparent to microwave, it can be used as reaction vessels and this property of microwaves, sharply reduce the amount of used organic solvent. The energy input is controlled so that the solvent and or the reaction mixture are not allowed to approach the boiling point too closely. The reduction of solvents and formation of lower amounts of by-products decrease the pollution at the source, shorten reaction time, use a few hundred grams scale and the lower energy consumption compared to conventional reaction condition (under reflux) are the advantage of green chemistry technique. A sample matrix such as a plant, which usually contains water as a component with high dielectric constant, because of the direct interaction of the
plant tissue with microwave, results in the subsequent rupture and releasing of the bio-organic compounds in to the organic solvent. For a system involving the usage of an organic solvent which absorb microwaves more strongly, such as ethanol, methanol and more effective heating of sample promoted by the dipole rotation of the reacting molecules could be achieved by increasing the microwave power output.

Recent developments and applications of modern sample-preparation techniques for the extraction, clean-up, and concentration of analyses from medicinal plant or herbal materials were reviewed (Hao et al., 2002; Kimbaris et al., 2006; Pan et al., 2003; Zhang et al., 2005; Huie, 2002). Commonly the effects of solvent or solvent composition, solvent volume, extraction temperature, and matrix characteristics (effect of sample granulometry, effect of on-line filtration of sample, and effect of moisture) for the optimization of the microwave-assisted extraction (MAE) process are studied. The assessment of these parameters seems to be the most important for plant materials. Recently in the developing countries, synthetic drugs are not only expensive and inadequate for the treatment of diseases but are also often with adulterations and side effects. Therefore the application of medicinal plants especially as traditional medicine is well acknowledged and established as a viable profession.

Nature has been a source of medicinal agents for thousands years and since the beginning of mankind. Extraction of bioactive compounds from medicinal plants has been permitted the demonstration of their physiological activity by medicinal researcher. Therefore there is need to search for plants with mediinally valuable and excellent extracts produced from widely varying substrates. Commiphora mukul is a small thorny plant indigenous to the India subcontinent and parts of the Near East (Mesorob et al., 1998). The ole-gum-resin of C. mukul is called gugglipid. The yellowish resin produced by the stem of the plant has been widely used in Ayurvedic medicine for more than 2000 years, mainly to treat arthritis and inflammation (Urizar and Moore, 2003; Kimmakkar et al., 2003). The active ingredients in gugglipid are the ketosteroids cis- and trans-4,17(20)-pregnadiene-3,16-dione, also known as E- and Z-guggulsterone (Ding and Staudinger, 2005), are extracted from the resin, that is safer and more effective than many cholesterol lowering drugs (Szapary et al., 2003).

The antimicrobial fraction of the methanolic extraction from the galls of Quercus infectoria is tannin. Tannins which are poly phenolic compounds and widely distributed in plants, foods, and beverages (Markkar and Becker, 1998), are soluble in water and polar organic solvents (Haslam, 1996). Their main characteristic is that they bind and precipitate (Min and Hart, 2003). Tannins raw hides into leather. But they have also been employed industrially in denaturing alcohol, in aqueous solution for treating burns and protecting plant against dehydration and damage by animals, as mordant on dyeing, as antidote for metallic, alkaloid and glycoside poisons, and as reagents in photography. They have been also added to mud in oil drilling operation to increase the viscosity and used for the manufacture of ink, rubber and plastics, preventing sculling in hot waters, for ores flotation and water treatment, and by combining them with binding agent such as phenol, formaldehyde to prepare adhesive (Åkaranța and Wankasi, 1999).

The galls of Q. infectoria, a commonly available plant in Iran were used as traditional medicine and studied pharmacologically (Hwang et al., 2004; Dar et al., 1976) obtained hydrolysable gallotannins by the solvent extraction of nutgalls (Regerat et al., 1989; Hwang et al., 2000). Cinnamomum verum J. S. Presl (synonym: Cinnamomum zeylanicum Blume, Cinnamom bark), mainly contains cinnamaldehyde, eugenol, trans cinnamic acid and phenolic compound, (Ranasinghe et al., 2003). The major active constituent of C. verum J. S. Presl extract is trans-cinnamaldehyde. It has various medicinal properties such as antipyretic, astringent, carminative, stomachic agents, antitumor, antibacterial and cytotoxic effect (Chang et al., 2001; Fang et al., 2004; Lee, 2002; Hussain et al., 1998).

In this work, much effort has been focused on the extraction of potentially useful products from the plant matrix by using MAE method and the results of extracted active compound were compared with those obtained by the reported conventional solvent extraction method at ambient temperature.

**MATERIALS AND METHODS**

The ole-gum-resin of C. mukul, the galls of Q. infectoria and the bark of C. verum J. S. Presl were purchased at the local market in Gorgan, Iran. The identities of plants were verified by comparing the collected specimens with those in the Gorgan University Herbarium. Standard E- and Z-guggulsterones were purchased from Steraloids, Newport, RI. All the chemicals used were of analytical grade or of the highest purity available, which were provided from Sigma-Aldrich and used as received. MAE experiments were performed on an Ethos 1600 (Figure 1). High Performance Liquid Chromatography (HPLC) system consisted of a diode array ultraviolet (UV) detector, a SC-04 (125 × 4.0 mm) PRONTOSIL 120-5-C18-H 5.0 μm column. Gas Chromatography (GC) was recorded by using Agilent Technology 5890 series II and column HP-5. Gas chromatography with mass-selective detector was used for analysis by Agilent GC/MS: HP 6890 N/5972N. Fourier Transform Infrared Spectroscopy (FT-IR) was performed on the product by using Perkin 100 spectrometer with KBr. 1H NMR spectra was recorded with a Bruker SF 250 spectrometer.

**Extraction and isolation E- and Z-guggulsterones from C. mukul**

The resin of C. mukul (13.3 g) was finely minced and extracted with EtOAc (ethylacetate) (130 mL) by using microwave irradiation (300 W, 80°C) under reflux condition for 1 h. After extraction, ole-gum resin was separated into two parts, gum and resin. The ethylacetate insoluble part of gum was chemically characterized as a carbohydrate gum (7.8 g) (belonging to the class of sugar). The
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Figure 1. Microwave setup (Ethos, 1600) and HEF 300/6 instalment.

resin contains bioactive components, especially E- and Z-guggulsterones which are soluble in EtOAc. After filtration and evaporation of the solvent under reduced pressure, 5.5 g compounds are separated. Fractionated in petroleum ether, the steroidal compounds were isolated by dissolving in petroleum ether (40 - 60°C). For isolation of E- and Z- guggulsterones from the mixture of steroidal compounds, after being evaporated of the solvent, 2.5 g of this was dissolved in small amount of EtOAc and transferred on to a silica gel 60F-254 column chromatography as stationary phase and eluted with the mixtures of solvents, toluene / acetone (9:1), which resulted in 15 fractions of 20 mL. Based on similar Thin Layer Chromatography (TLC) aluminium sheet (20 × 20) profiles, four fractions (8 - 11) were combined (Rs value for E: 0.36 and Z: 0.44) (Himani et al., 2004). For identification and quantitative analysis of these isomers in herbal extracts, the HPLC technique with photo diode array detector and gradient solvent was applied. At first, the calibration curve for E- and Z-guggulsterones was developed. The guggulsterones peaks were identified at 242 nm on symmetry C18 steel reversed-phase column (SC-04: 125 × 4.0 mm, 5.0 µm particle size). The mobile phase was acetonitrile / water (46 / 54 v/v), the system was operated at ambient temperature (~ 28°C) and a flow rate of 1.0 mL/min. A valid quantitative range for E- and Z-guggulsterones with high precision in guggulipid, was evaluated by a calibration standard curve.

Preparation of standard and sample solution

Standard solution was prepared by dissolving each E- and Z-guggulsterone (0.6 mmol) in 25 mL acetonitrile separately. Calibration standard solutions were prepared by the dilution of stock standard solution with acetonitrile in concentration range of 0.1 - 0.33 mmol/L for E-guggulsterone and 0.1 - 0.5 mmol/L for Z-guggulsterone. All standard solutions were filtered through 0.45 µm pore-size membrane filter before injection. The sample solution was prepared by transferring the sample with equivalent of 0.0103 g in 10 mL acetonitrile, and then a portion of it was filtered before injection into HPLC. The linear regression analysis of the data for the calibration plots of E- and Z-guggulsterone showed good linear relationship with \( r^2 = 0.9994 \) and 0.9954 respectively, in the concentration rate of 0.1 - 0.5 mmol/L spots.

Extraction and isolation of Tannin from the galls of Q. infectoria

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The dried and powder galls (15.3 g) of Q. infectoria was placed in two neck balloon (250 mL) and extracted with MeOH (methanol) (125 mL) at 67°C for 30 min under microwave irradiation. The extracted product was filtrated and solvent evaporated in a vacuum rotary evaporator at 50°C. After being removed the solvent, 8.3 g gummy product was produced. 50 mL Et₂O (diethylether) was added to the extracted product, a pale yellow (2.8 g) powder precipitated. After filtration, the separated compound (tannic acid) was analyzed by FT-IR and \(^1H\) NMR spectroscopy. Tannic acid is a light to brown amorphous granular powder with the chemical formula of \( \text{C}_{45}\text{H}_{52}\text{O}_{10} \) which decomposes at 210 - 215°C. It is soluble in water, acetone and ethanol, insoluble in benzene, chloroform and ether. A solution of the sample after addition of a small quantity of ferric chloride, a bluish black colour or precipitate formed. The identification and quantitative analysis of tannic acid was performed by reversed-phase HPLC on a C18 column by using a binary gradient elution with mobile phases consisting of an aqueous methanolic eluents at low pH. The gradient system consisted of solvent A (25 mL acetic acid and 975 mL distilled water) and solvent B (99.8% methanol) pumped at 1 mL /min. The gradient started with 100% solution A and ended with 100% solution B at 30 min. A solution of 1 g gummy product in 100 mL of absolute ethanol was injected at a volume of 20 µL and detected at the maximum wavelength of tannic acid (280 nm). The column temperature was maintained at 30°C. The sample peaks were identified by comparing with the standard solution of tannic acid. The percentage of tannic acid in the extracted product was determined from the appropriate calibration curves. The correlation coefficient of the calibration curve of 0.1, 0.2, 0.4, 0.6, 0.8 and 1 mmol/L of standard tannic acid in absolute ethanol as above gradient elution was greater than 0.993.

Extraction and isolation of trans-cinnamaldehyde from the bark of C. verum J. S. Presl:

In each of the inner glass vials, 3 g dried and finely powdered of the bark from Cinnamomum cassia and a magnetic stir bar were placed
RESULTS AND DISCUSSION

In our previous studies, we found that, MW extraction technique under the optimal condition compared to the conventional extraction methods such as solvent extraction; Supercritical fluid extraction, soxhlet extraction and ultrasound extraction for the extraction of bioactive compounds from the medicinal plants are more effective. We have also been evaluated that, the efficiency of microwave energy was strongly dependent on the physiochemical properties both of the solvent and the solid matrix. Based on the obtained results, parameters such as sampling time, temperature, microwave irradiation power and desorption time also affected the microwave-assisted extraction of plant materials with medicinal significance (Ondruschka and Asghari, 2006). In our research work, we hope that this article provide to progresses of the existing methodologies MAE in the extraction of bioactive compound from the medicinal plants which were provided from the Iranian markets such as E- and Z- Guggulsterone (Figure 2) from the C. mukul, tannin from the galls of Q. infectoria and cinnamaldehyde from the C. verum J. S. Presl under the optimal condition and comparison with results obtained with Conventional Solvent Extraction (CSE) method. The extracted products were isolated and identified by column chromatography, UV spectroscopy, GC, HPLC, GC-Mass spectrometry, FT-IR and $^1$H-NMR.

With regard to the quantification of E- and Z-guggulsterone by using HPLC spectroscopy analysis a valid quantitative range of E- and Z-guggulsterones in guggulipid was evaluated (2.5 - 3%) by a calibration standard curve. The signal at $R_f$ =11.94 min was identified E- Guggulsterone, and second signal at $R_f$ = 16.89 min was identified Z- Guggulsterone with help of the chromatograms of their individual standard isomers (Figure 3). Interestingly, in the total ketonic fraction (53% w/w) the Z-isomer of guggulsterone contributes almost 80% (w/w) of total guggulsterone in herbal extract respectively, whereas in case of standard guggulsterone mixture, the amount of Z- isomer is 45% (w/w) of total guggulsterone. It may be extended to study the degradation of guggulsterone under different stress condition. Analysis of crude product by GC and GC-MS shows approximately 90% stereo Z- and 10% E-guggulsterone. Z- and E-guggulsterones were eluted at 37.65 and 40.45 min, respectively (Figure 4).

An antibacterial compound from the galls of Q. infectoria extraction is tannic acid. Based on the HPLC spectroscopy analysis, total tannic acid content was found to be 10 - 20% w/w of the extract with $R_f$ = 1.86 min (Figure 5). The structure of tannic acid was confirmed by the spectroscopic data (Figures 6 and 7). Extraction of tannic acid from Q. infectoria at room temperature by conventional method such as soxhlet apparatus needs a time as long as 24 h (Hwang et al., 2000). HEF method,
at 300 W for 1 h at 120°C was used for extraction of trans-cinnamaldehyde from C. verum J. S. Presl. The extracted product was observed by GC-Mass analysis at $R_f = 7.6$ min, confirming the structure of cinnamaldehyde. For the quantitative determination of cinnamaldehyde, HPLC we used at the maximum absorption wavelength of cinnamaldehyde. The signal of cinnamaldehyde was observed around 18.3 min after injection of the sample (Figure 8). Cinnamaldehyde (0.84 - 1.0%) was obtained in the extraction of cinnamomum bark.

**Optimization of microwave extraction**

Several variables could potentially affect the extraction efficiency including temperature, time and solvent and microwave irradiation power. However, the effects of extraction temperature and polarity of solvent were significant and showed a positive effect on MAE. In the open vessels equipped by the condenser used for the extraction of E-and Z-guggolsterone from C. mukul and tannin from Q. infectoria, system works at the atmospheric pressure, and the maximum temperature was determined by the boiling point of the solvent used. The solvent is heated and refluxed through the sample, and in this case the microwave irradiation is focused on the sample in vessel which allows homogeneous and very efficient heating. Extraction of cinnamaldeyde from Cinnamomum bark was conducted in closed vessel in which temperature may reach well above the boiling point of the solvent. This elevated temperature does indeed result in improved extraction efficiencies since desorption of analyte from active sites in the matrix would be increased. In terms of the solubility, decreasing the polarity of the extraction solvent e.g. EtOAc, MeOH as less polar solvent led to the enhancement of the solubility of biologically active compounds in the plant matrix C. mukul and Q. infectoria respectively. EtOAc as compared

**Figure 3.** HPLC profile of E- and Z-guggulsterones from the C. mukul (guggul) resin extract. Elution with CH$_3$CN/ H$_2$O, 46:54 (v/v) as mobile phase with photo diode array detector at 242 nm.
Figure 4 (i). HPLC profile of E- and Z-guggulsterones in the standard mixture.

Figure 4 (ii). HPLC profile of standard Z-guggulsterone.
Figure 4 (iii). HPLC profile of standard E–guggulsterone.

Figure 4 (iv). Gas chromatogram of microwave-assisted extraction of guggulsterones from the resin of C. mukul.
Figure 5 (i). Gas chromatogram of E- guggulsterone standard and its mass spectrum

Figure 5 (ii). Gas chromatogram of Z- guggulsterone standard and its mass spectrum.
Figure 5 (iii). Gas chromatogram of Z- guggulsterone standard and its mass spectrum

Figure 5 (iv). HPLC profile of tannic acid extracted from Q. infectoria.
Comparison of conventional and microwave-assisted extraction methods

Ordinary solvent extraction at room temperature was carried out in order to compare MAE with a traditional extraction method (Table 1). The main advantages of the use of MAE are the considerable reduction of time, solvent consumption and increased purity of crude extracts when compared to conventional extraction.
In conclusion, we find the use of microwave-assisted extraction leads to very fast extraction rate with high value of compounds compared to the steam distillation and solvent extraction techniques. Furthermore, there is a facility in the separation, increased purity of crude extracts from the matrix plant in the MAE methods.

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### Table 1. Comparison microwave-assisted extraction with the conventional extraction methods.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Plant</th>
<th>C. mukul</th>
<th>Q. infectoria</th>
<th>C. verum J.S.Presl</th>
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<td>Extraction solvent (mL)</td>
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<td>CSE a</td>
<td>MAE</td>
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a soxhlet, b steam distillation, c mg isolated active compound/g of dry plant material.

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