Comparisons of various extraction techniques for the determination of chemical constituents *Lycium barbarum* fruit (Goji)

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Abstract

Lycium barbarum (Goji berry, Wolfberry, or *Gougi*) has been use for a long time as an ingredient in Chinese cuisine and brewing, and also in traditional Chinese herbal medicine. The objective of this study was to compare various extraction techniques, Supercritical cabon dioxide extraction, Soxhlet extraction and Microwave extraction, for determination of chemical constituents extracted from *L. barbarum* fruit (Goji) using Gas Chromatography-Mass Spectrometry (GC-MS) analysis. The highest extraction yield was 20% by Supercritical carbon dioxide extraction method. Extraction was carried out under the pressure of 4,000 psi, temperature range of 40-50°C for 3 hrs. The result of GC–MS analysis, the retention time, mass data and area percentage (concentration) were reported. The major components of extraction were hexadecanoic acid (10.47%), linoleic acid (9.41%), 9-octadecenoic acid (Z) (7.18%), 9,12-octadecadienoic acid (Z,Z) (6.87%) and octadecenoic acid (2.75%). AS the result, the main components of *L. barbarum* fruit were essential oil and fatty acids.

Keywords: *Lycium barbarum*, Supercritical cabon dioxide extraction, Soxhlet extraction, Microwave extraction, GC-MS analysis

1. Introduction

Chinese Wolfberry (*Lycium barbarum*), also known as Goji berry, is a Solanaceous defoliated shrubbery that grows in China, Tibet and other parts of Asia. Its fruits are 1–2 cm long, bright orange -red ellipsoid berries, which has a long history of use in Chinese and Indian medicine. A ripe fruit has been used in Asian countries as a traditional herbal medicine.

The Chinese wolfberry is a well-known Chinese herbal medicine as well as a tonic which has been used in East Asia for thousands of years to treat and prevent diseases such as insomnia, liver dysfunction, diabetes, visual degeneration, and cancer. People in such area believe that wolfberry fruit has many health benefits and use them to make tea, soup, stew and wine or chew them like raisins [1].

Most of the wolfberry fruits are dried using sun light, Dried berries are consumed as a usual part of the Chinese diet and are a common ingredient in commercial food products, supplements, and traditional Chinese medicine. Wolfberry is frequently added to soups, hot pots, and herbal teas, and is also popularly soaked in wines alone or together with other traditional Chinese medicine ingredients to make functional wines [2].

Fig. 1. *Lycium barbarum* fruit
Source **:** Amagase and Farnsworth [3]

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The Chinese wolfberry contains various vitamins A, B-1, B-2, B-5, C and E. Additionally, the Chinese wolfberry has protein, essential fatty acids including polyunsaturated fats, and carbohydrates. The carbohydrates in this berry are complex carbohydrates that are essential for sustainable energy. Further, the berry offers polysaccharides and dietary fiber. The minerals available in Chinese wolfberry include iron, phosphorus, copper, calcium, germanium, magnesium and zinc.

Solvent extraction is one of the well-known traditional separation methods since Palaeolithic age. The science of solvent extraction has evolved over a long period of time and much progress has been made in the understanding of solvation and the properties of liquid mixtures used in extraction processes [4].

A supercritical fluid extraction (SFE) is an extraction carried out using a supercritical fluid. High demand for good quality oils urged researchers to find safer techniques for the extraction of the desired components while at the same time reduced thermal degradation and solvent contamination to a minimum [5].

Soxhlet extraction is one of the oldest techniques for isolating metabolites from natural material. The technique is used for the isolation and enrichment of analytes of medium and low volatility and thermal stability. It allows a high recovery, but has a number of shortcomings, including long extraction time and large consumption of solvents, cooling water and electric energy. This extraction is now in common use, being applied to the determination of, among others, lipids and polycyclic aromatic hydrocarbons in natural products (e.g., coffee, soybean and coconut oil, mushrooms, fruits, and vegetables). Soxhlet extraction was also used in investigations of antiinflammatory and antibacterial properties of plant metabolites. Nine African plants were examined and their therapeutical properties determined on the basis of pharmacological properties of the extracts [6].

Microwave-assisted extraction (MAE) or simply microwave extraction is a relatively new extraction technique that combines microwave and traditional solvent extraction. Application of microwaves for heating the solvents and plant tissues in extraction process, which increases the kinetic of extraction, is called microwave-assisted extraction. MAE has a number of advantages, e.g., shorter extraction time, less solvent, higher extraction rate and lower cost, over traditional method of extraction of compounds from various matrices, especially natural products. [7].

The aim of the present study was to compare of various extraction techniques, Supercritical cabon dioxide extraction, Soxhlet extraction and Microwave extraction for the determination of chemical constituents *L. barbarum* fruit (Goji) by Gas Chromatograph-Mass Spectrometer (GC-MS) analysis. The constituents identified by GC-MS analysis, their retention time, mass data and area percentage (concentrations) was reported here.

2. Materials and Methods

2.1 Plant materials and Chemical reagents

L. barbarum fruit was collected from Ningxia region of China in 2012, and the fruits were lyophilized, and finely powdered. The following chemical reagents were used ethyl ether, n-hexane, acetone, 95% ethanol, anhydrous sodium sulfate, carbon dioxide and nitrogen.

2.2 Extraction Methods

2.2.1 Soxhlet extraction

10 g and 20 g of dried sample were weighed, then transfered to the cellulose thimbles. A wad of cotton was placed on top of the sample to minimize streaming and channeling through the sample. The thimbles were loaded into the main chamber of the Soxhlet extractor. The samples were extracted with 200 mL of ethyl ether at 55°C for 8 hr and 200 mL of *n*-hexane at 85°C for 8 hrs. (Table 1). After extraction completed, the solvents were removed at 50°C under reduced pressure using a rotary evaporator. Non-soluble portion of the extracted solid remains in the thimbles, and is usually discarded. After evaporation of ethyl , the extract were dried under an N_2 stream. Then, the extract were preserved at 4°C until analysis.

Table 1. Physical values of control factors corresponding of Soxhlet extraction.

Run	Sample size (g)	Solvent	Time (hr)	Temperature (°C)
1	10	ethyl ether	8	55
2	20	ethyl ether	8	55
3	30	*n*-hexane	8	85
4	40	*n*-hexane	8	85

2.2.2. Microwave extraction

Microwave extraction was performed in the closed vessel unit MARs X-press (CEM, Matthews, NC28106, USA & Canada). 5 g of dried sample was weighed and transferred to the 6 extraction vessels and was added 40 ml of an *n*-hexane–acetone (1:1) mixture overall extraction vessels. Extraction vessels were put in vessel holder and loaded into the microwave extractor. The extraction was carried out under microwave power of 100%, extraction temperature of 105°C, extraction pressure of 45 psig, extraction time of 8 minutes and extraction cycle of 1,3 and 5 (Table 2). After extraction finished, the solvent was filtered through Whatman No.1 filter paper and was

removed at 40°C under reduced pressure using a rotary evaporator and the extract were dried under an N_2 stream. Then, the extracts were preserved at 4°C until analysis.

A 5 g of dried sample was weighed and transferred to the 6 extraction vessels and was added 40 ml of ethyl ether overall extraction vessels. Extraction vessels were put into vessel holder and loaded into the microwave extractor. The extraction was carried out under microwave power of 100%, extraction temperature of 100°C, extraction pressure of 90 psig, extraction time of 8 minutes and extraction cycle of 1,3 and 5 (Table 2). After extraction finished, the solvent was filtered through Whatman No.1 filter paper and was removed at 35°C under reduced pressure using a rotary evaporator and the extract were dried under an N_2 stream. Then, the extracts were preserved at 4°C until analysis.

2.4. Gas Chromatograph - Mass Spectrometry (GC-MS) analysis of chemical composition

The samples were also analysed on an Agilent GC–MS system was a 5973N mass-selective detector (MSD) equipped with HP6890A Series Injector. The MSD was operated in the electron ionization mode at 70 eV and the acquisition mode was scan mode. The GC capillary column was HP-5MS (0.25 mm×30 m internal diameter, with a film thickness of 0.25 μm) and the oven temperature was programmed as follows: initial temperature of 60°C and then increased to 200 °C by 5 °C/min and, finally, increased to 230 °C by 2 °C/min and held for 10 min. The temperature of the injection port and detector interface was set at 270°C and 280°C, respectively. Helium was used as the carrier gas at a constant column flow rate of 1 ml/min. A 0.5 μL of the sample extract was injected in the splitless mode. The analyzes were identified by autoscan and direct comparison of their mass spectral pattern and retention index with the WILEY275 Mass Spectral Database.

Table 2. Physical values of control factors corresponding of Microwave extraction for this study.

Run	Solvent	Pressure (psig)	Time (min)		Temperature (°C)	Extraction cycle (step)
			Ramp	Hold		
1	*n*-hexane–acetone (1:1)	45	8	10	105	1
2	*n*-hexane–acetone (1:1)	45	24	20	105	3
3	*n*-hexane–acetone (1:1)	45	40	30	105	5
4	ethyl ether	90	8	10	100	1
5	ethyl ether	90	24	20	100	3
6	ethyl ether	90	40	30	100	5

2.2.3. Supercritical carbon dioxide extraction

Extraction assay was conducted as described by Guoliang *et al*. [8]. Supercritical carbon dioxide extraction treatment: in all experiments, 5 g samples of prepared *L. barbarum* fruits were placed in the extractor cylinder and after an initial air purge, liquefied CO_2 was pumped into the vessel and consequently the pressure was raised to 4,000 psi, temperature of 40°C, 45°C and 50°C for 3 hrs. (every one hour – weight out). After extraction finished, the extract were dried under an N_2 stream. Then, the extracts were preserved at 4°C until analysis.

2.3. Sample preparation for analysis

The highest crude extraction yield of Soxhlet extraction were 3.33 % and 2.50% in Table 1 (Run 1 and Run 4), repectively, extraction yield of Microwave extraction were 2.87 % and 5.81% in Table 4 (Run 3 and Run 5), repectively and extraction yield all of Supercritical carbon dioxide extraction were 20% in Table 5. The sample extract from the highest crude extraction yield of Soxhlet extraction, Microwave extraction and Supercritical carbon dioxide extraction were dissolved with *n*- hexane and dried by anhydrous sodium sulfate and stored at 4°C until GC–Mass Spectrometry (MS) analysis.

2.5. Scanning Electron Microscope analysis

In this study, scanning electron microscopy (SEM) microphotography was used for comparison of the morphology of *L. barbarum* fruit before and after extraction by Supercritical carbon dioxide extraction method under the pressure of 4,000 psi, temperature of 45°C for 3 hrs. The samples were then coated with gold in a coater (E-1010, Hitachi, Japan) and investigated on a scanning electron microscope (Model S-3000N, Hitachi, Japan).

3. Result and Discussions

3.1. Chemical constituents of *L. barbarum*

3.1.1. Soxhlet extraction with ethyl ether

The chemical constituents of *L. barbarum* fruit extracted with ethyl ether were analyzed by gas chromatography-mass spectrometry (GC-MS) (Fig. 2). Sixty-six components, representing 72.28% of the total extract

were identified. The major components of chemical compounds were hexadecanoic acid (9.81%), linoleic acid (9.41%), butylated hydroxy toluene (7.02%), 9-octadecenoic acid (Z) (5.56%) and octadecanoic acid (2.53%).

The constituents identified by GC-MS analysis, retention times : (19.35) butylated hydroxy toluene, (26.14) isopropyl myristate, (29.57) hexadecanoic acid, ethyl ester, (30.23) hexadecanoic acid, (33.90) octadecenoic acid, (34.79) 9-octadecanoic acid (Z) and (46.03) docosanoic acid.

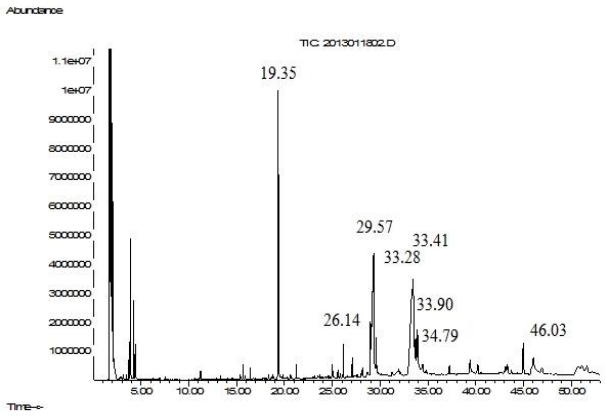

Fig. 2. GC-MS Analysis of Chemical constituents in the sample extracted from *L. barbarum* fruit by Soxhlet extraction with ethyl ether.

3.1.2. Soxhlet extraction with *n*-hexane

The chemical constituents of *L. barbarum* fruit extracted with *n*-hexane were analyzed by gas chromatography-mass spectrometry (GC-MS) (Fig. 3). Sixty-five components, representing 71.72% of the total extract were identified. The major components of chemical compounds were heptadecanoic acid (9.98%), linoleic acid (8.44%), 9-octadecenoic acid (Z) (7.18%) and octadecanoic acid (2.14%).

The constituents identified by GC-MS analysis, retention times : (11.22) dodecane, (16.43) n-tetradecane, (21.24) hexadecane, (25.59) octadecane, (29.26) heptadecanoic acid, (33.28) linoleic acid, (33.41) 9-octadecanoic acid (Z), (33.86) octadecenoic acid and (41.24) docosanoic acid.

3.1.3. Microwave extraction with ethyl ether

The chemical constituents of *L. barbarum* fruit extracted with ethyl ether were analyzed by gas chromatography-mass spectrometry (GC-MS) (Fig.4). Forty components, representing 31.38% of the total extract were identified. The major components of chemical compounds were hexadecanoic acid (2.73%), 9-octadecenoic acid (Z) (2.37%) and 9,12-octadecadienoic acid (Z,Z) (1.83%).

The constituents identified by GC-MS analysis, retention times : (11.22) dodecane, (16.43) tetradecane, (19.37) 2,4-di-tert-butylphenol, (21.23) hexadecane, (25.59) octadecane, (29.48) hexadecanoic acid, (33.09) 9,12-octadecadienoic acid (Z,Z), (33.22) 9-octadecanoic acid (Z), (33.74) octadecenoic acid and (46.03) docosanoic acid.

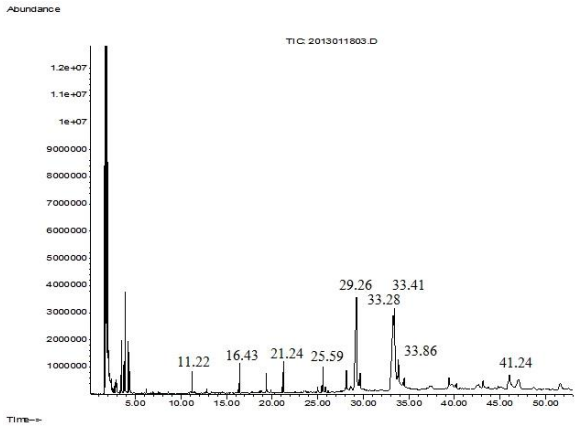

Fig. 3. GC-MS Analysis of Chemical constituents in the sample extracted from *L. barbarum* fruits by Soxhlet extraction with *n*-hexane.

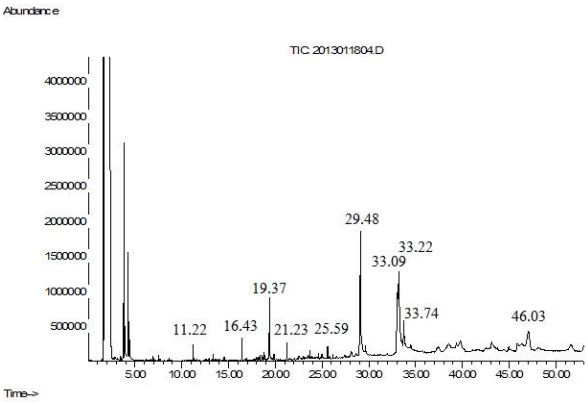

Fig. 4. GC-MS Analysis of Chemical constituents in the sample extracted from *L. barbarum* fruits by Microwave extraction with ethyl ether.

3.1.4. Microwave extraction with ethyl ether

The chemical constituents of *L. barbarum* fruit extracted with ethyl ether were analyzed by gas chromatography-mass spectrometry (GC-MS) (Fig.5). Forty components, representing 31.38% of the total extract were identified. The major components of chemical compounds were hexadecanoic acid (2.73%), 9-octadecenoic acid (Z) (2.37%) and 9,12-octadecadienoic acid (Z,Z) (1.83%).

The constituents identified by GC-MS analysis, retention times : (11.22) dodecane, (16.43) tetradecane, (19.37) 2,4-di-tert-butylphenol, (21.23) hexadecane, (25.59) octadecane, (29.48) hexadecanoic acid, (33.09) 9,12-octadecadienoic acid (Z,Z), (33.22) 9-octadecanoic acid (Z), (33.74) octadecenoic acid and (47.06) docosanoic acid.

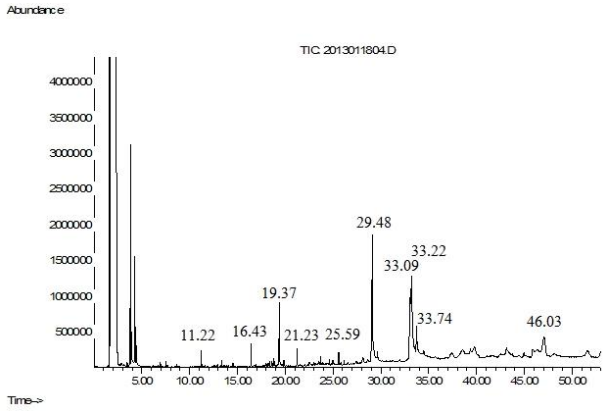

Fig. 5. GC-MS Analysis of Chemical constituents in the sample extracted from *L. barbarum* fruits by Microwave extraction with ethyl ether.

3.1.5. Microwave extraction with *n*-hexane-acetone (1:1)

The chemical constituents of *L. barbarum* extracted with *n*-hexane-acetone (1:1) were analyzed by gas chromatography-mass spectrometry (GC-MS) (Fig. 6). Fifty-two components, representing 33.32% of the total extract were identified. The major components of chemical compounds were hexadecanoic acid (5.31%), 9-octadecenoic acid (Z) (3.21%), Eicosane (2.11%) and octadecanoic acid (1.76%).

The constituents identified by GC-MS analysis, retention times : (10.08) 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one, (11.22) dodecane, (16.43) tetradecane, (19.38) phenol, 2,4-bis(1,1-dimethylethyl), (21.24) hexadecane, (25.59) octadecane, (29.24 hexadecanoic acid, (33.34), (33.21) linoleic acid 9-octadecanoic acid (Z), (33.84) octadecenoic acid and (46.00) docosanoic acid.

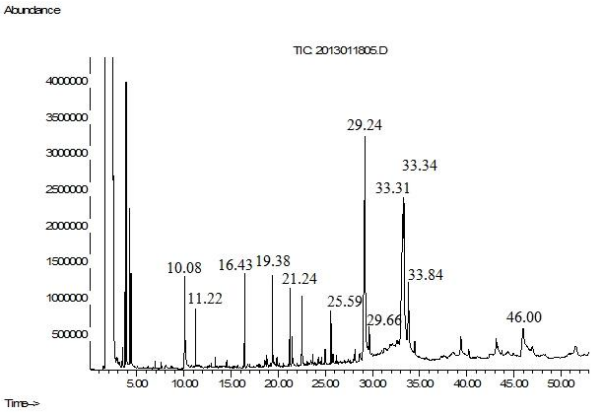

Fig. 6. GC-MS Analysis of Chemical constituents in the sample extracted from *L. barbarum* fruit by Microwave extraction with *n*-hexane-acetone (1:1).

3.1.6. Supercritical CO_2 extraction at 40°C

The chemical constituents of *L. barbarum* fruit by Supercritical CO_2 extraction at 40°C was analyzed by gas chromatography-mass spectrometry (GC-MS) (Fig. 7). Sixtysix components, representing 25.03% of the total extract were identified. The major components of chemical compounds were hexadecanoic acid (9.18%) bis-(octylphenyl)-amine (5.50%) and phenol, 2,4-bis(1,1-dimethylethyl) (0.94%).

The constituents identified by GC-MS analysis, retention times : (16.42) tetradecane, (19.36) phenol, 2,4-bis(1,1-dimethylethyl), (21.04) 1-hexadecane, (29.27) hexadecanoic acid and (51.92) bis-(octylphenyl)-amine.

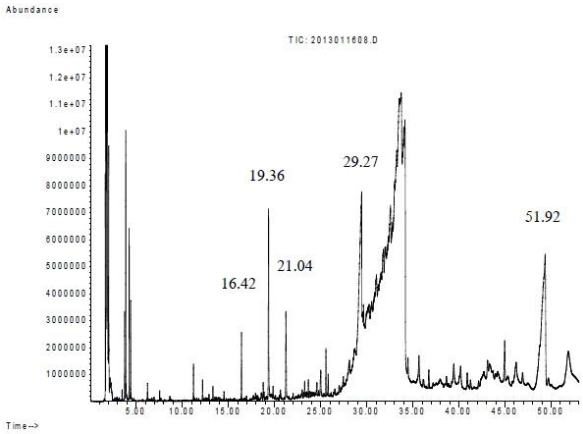

Fig. 7. GC-MS Analysis of Chemical constituents in the sample extracted from *L. barbarum* fruit by Supercritical CO2 extraction at 40°C.

3.1.7. Supercritical CO_2 extraction at 45°C

The chemical constituents of *L. barbarum* fruit extracted at 45°C were analyzed by gas chromatography-mass spectrometry (GC-MS) (Fig. 8). Sixtyfive components, representing 57.51% of the total extract were identified. The major components of chemical compounds were hexadecanoic acid (9.18%), 9,12-octadecadienoic acid (Z,Z) (6.87%), linoleic acid (3.81%), 9-octadecenoic acid (Z), (3.28%) and octadecanoic acid (2.75%).

The constituents identified by GC-MS analysis, retention times : (16.46) tetradecane, (19.42) phenol, 2,4-bis(1,1-dimethylethyl), (21.02) 1-hexadecane, (29.18) hexa-decanoic acid, (33.67) linoleic acid, (33.68) 9-octadecenoic acid (Z) and (48.50) bis-(octylphenyl)-amine.

3.1.7. Supercritical CO_2 extraction at 50°C

The chemical constituents of *L. barbarum* fruit extracted at 50°C were analyzed by gas chromatography-mass spectrometry (GC-MS) (Fig. 9). Seventy-eight components, representing 59.67% of the total extract were identified. The major components of chemical compounds were hexadecanoic acid (10.47%), linoleic acid (7.76%),

9-octadecenoic acid (Z) (3.78%), bis-(octylphenyl)-amine (2.75%) and octadecanoic acid (1.76%).

The constituents identified by GC-MS analysis, retention times : (16.39) tetradecane, (19.29) phenol, 2,4-bis(1,1-dimethylethyl), (21.01) 1-hexadecane, (29.20) hexadecanoic acid, (33.24) linoleic acid, (33.38) 9-octadecenoic acid (Z), (33.81) hexadecanoic acid, (45.91) docosanoic acid and (48.28) bis-(octylphenyl)-amine.

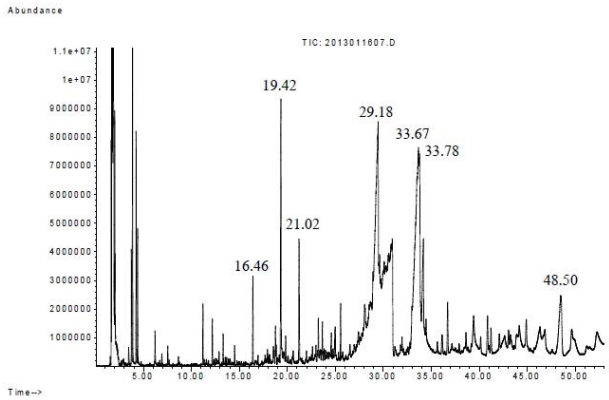

Fig. 8. GC-MS Analysis of Chemical constituents in the sample extracted from *L. barbarum* fruit by Supercritical CO_2 extraction at 45°C.

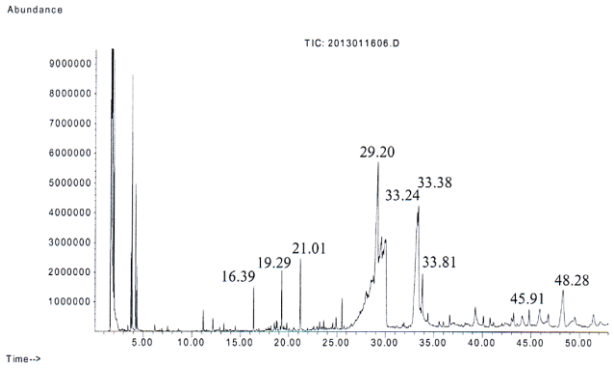

Fig. 9. GC-MS Analysis of Chemical constituents in the sample extracted from *L. barbarum* fruit by Supercritical CO_2 extraction at 50°C.

3.2. Comparisons of chemical compounds

The chemical compounds of *L. barbarum* fruit from Soxhlet extraction with ethyl ether and *n*-hexane were analyzed by gas chromatography-mass spectrometry (GC-MS). Thirty components were same chemical compounds. The major components of chemical compounds were hexadecanoic acid, lino leic acid, 14-methyl-8-hexadecyn-1-ol, 9- octadecenoic acid (Z), octadecanoic acid, docosane, eicosane and docosanoic acid.

The chemical compounds of *L. barbarum* friut from Microwave extraction with ethyl ether and *n*-hexane-acetone (1:1) were analyzed by gas chromatography-mass spectrometry (GC-MS). Twenty-three components were same chemical compounds. The major components of chemical compounds were hexadecanoic acid, 9,12-octadecadienoic acid (Z,Z), 9- octadecenoic acid (Z), octadecenoic acid and nonacosane.

The chemical compounds of *L. barbarum* fruit from Supercritical CO_2 extraction at 40°C 45°C and 50°C were analyzed by gas chromatography-mass spectrometry (GC-MS). Twenty-three components were same chemical compounds. The major components of chemical compounds were hexadecanoic acid, linoleic acid, 9- octadecenoic acid (Z), 9,12-octadecadienoic acid (Z,Z) and octadecenoic acid.

Kim *et al.* [9] reported that the major components found in dried fruit of *L. chinensis* Miller were hexadecanoic acid, 9,12-octadecadienoic acid (Z,Z), 3-methylbutanal, 2-methyl butanal 62 and 2-furancarboxal dehyde. The essential oil and fatty acids of *L. barbarum* and *L. rutinicum* have been reported (Altintas *et al*., 2006). The main components in the essential oil of *L. barbarum* were hexadecanoic acid, linoleic acid, **β**-elemene, myristic acid and ethyl hexadecanoate. The essential oil of *L. ruthenicum* has heptacosane, ethyl linoleate, hexacosane, and nonacosane and ethyl hexadecanoate have been identified as the main constituents [10].

The essential oil composition of the fruits of *L. chinensis* has previously been reported, with ethyl hexadecanoate, 1-octadecanone, tetrapyrazine,2-furan-carboxaldehyde, and ethyl linoleate as the main constituents [11].

The essential oil composition of the fruits of *L. chinensis* has previously been reported, hexadecanoic acid, hexadecanoic acid ethyl ester, hexadecanoic acid methyl ester , ethyl linoleate, and phytol as the main constituents [12].

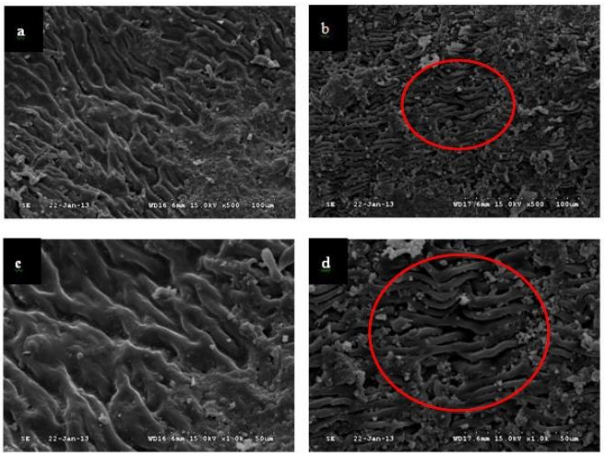

Fig. 10. Scanning electron microscopy of *L. barbarum* fruit skin (texture of surface). Before extracted by Supercritical carbon dioxide extraction method(a), (c). After extracted by Supercritical cabon dioxide extraction method (b), (d).

3.3. Scanning Electron Microscope

Fig. 10 and Fig. 11. shows morphology of *L. barbarum* fruit before and after extraction by Supercritical carbon dioxide extraction method. The condition was under the pressure of 4,000 psi, temperature range of 45°C for 3 hrs. After extracted texture of surface and cross section of *L. barbarum* fruit were broken and shriveled by Supercritical carbon dioxide.

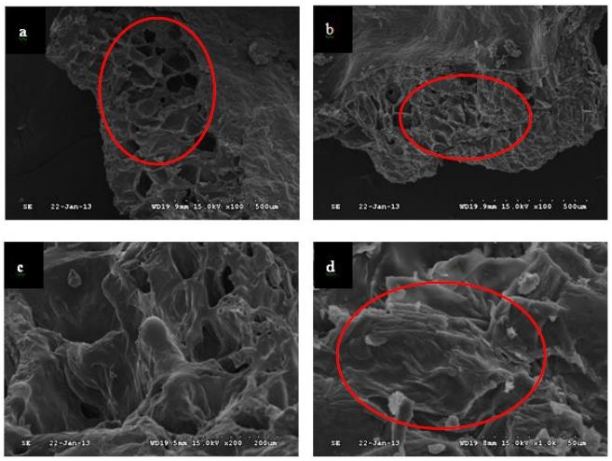

Fig. 11. Scanning electron microscopy of *L. barbarum* fruit (cross section). Before extracted by Supercritical cabon dioxide extraction method (a), (c). After extracted by Supercritical cabon dioxide extraction method (b), (d).

4. Conciusion

In this study, comparisons of various extraction techniques, of chemical constituents from *L. barbarum* fruit were exhibited. The highest extraction yield was 20% by Supercritical carbon dioxide extraction method. The extraction was carried out under the pressure of 4,000 psi, temperature range of 40-50°C for 3 hrs. The result of Gas Chromatography-Mass Spectrometry (GC-MS) analysis show the 5 major components of chemical compounds were hexadecanoic acid, linoleic acid, 9- octadecenoic acid (Z), 9,12-octadecadienoic acid (Z,Z) and octadecenoic acid. As the result, it was found that the main components of *L. barbarum* fruit were found to be essential oil and fatty acid which are important for food industrial applications. Our results indicate that the viability of Supercritical carbon dioxide extraction for food industrial applications.

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