Characteristics of ginseng seed oil prepared by different extraction methods and its whitening effect

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Introduction:

As a well-known medicinal plant, ginseng is the most widely used as botanical cosmetic raw materials around the world, especially in East Asia. Most of the research on ginseng focuses primarily on its roots. With the advancement of science and technology and people's yearning for health, in recent years, more in-depth research has been done on the medicinal ingredients and health care value of the roots, stems, leaves and other parts of ginseng, whereas the seeds remain poorly understood[1-2].

In recent years, demand for seed oils as ingredients for food, cosmetics and biofuel has greatly increased as industry seeks natural alternatives, creating pressure on countries providing the raw material to meet the growing demand[3-4]. In comparison with the ginseng root, the seed was always neglected in commercial terms. Actually ginseng seeds are rich in oil, and their unsaturated fatty acid content exceeds that of olive oil[5-7]. The special ratio of unsaturated fatty acids indicated that ginseng seed oil (GSO) had good utilization prospects. As ginseng producers, China has rich resources of ginseng, advantaged superiority condition compared with other countries. Therefore, it is of great significance to seek and extract functional substances from ginseng for the research and development of ginseng cosmetics and the promotion of the development of ginseng industry. The purpose of this research is to screen the appropriate extraction process for ginseng seed oil and to evaluate its effect on skin whitening, expecting to provide a basis for the in-depth development of ginseng seed oil and lay a foundation for its application in cosmetics.

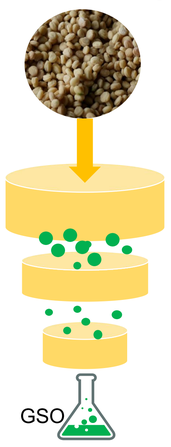
Materials & Methods:

Materials

The ginseng seeds used in this study were provide by Fusong Natural LvBao Plant Science and Technology Co., Ltd. And the whole ginseng seeds were crushed in a small medicinal material crusher prepared for supercritical fluid extraction and solvent extraction.

Extraction Methods

Preparation of ginseng seed oil



- **Cold pressing extraction**

Ginseng seed endosperm was pressed to obtain crude oil which was deposited overnight at 4℃, then centrifuged eliminate impurities.

- **N-hexane code extraction**

The ginseng seed powder and n-hexane were added according to the ratio of solid to liquid of 1:2, and soaked overnight at room temperature. After filtration, the filter residue was soaked in n-hexane for 8h and repeated twice. The filtrate was combined and the solvent was removed by rotary evaporation to obtain cold extracted ginseng seed oil.

- **N-hexane thermal extraction**

Same as the n-hexane code extraction, but the extraction temperature was carried out at 65℃ for 2h repeated 3 times.

- **Ethanol extraction**

The ginseng seed powder and 95% ethanol were added according to the ratio of solid to liquid of 1:2, and soaked overnight at room temperature then reflux extraction was carried out at 65℃ for 2h, reflux extraction was carried out for 2 times, filtrate was combined, and solvent was removed by rotary evaporation.

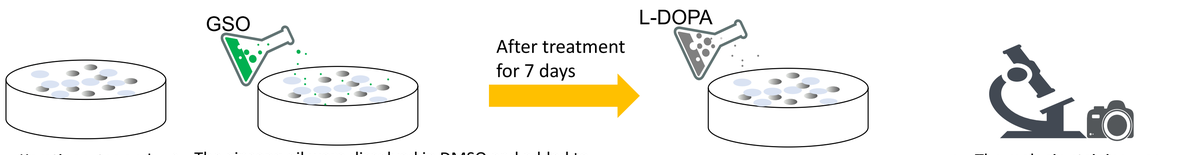
- **Supercritical CO_2 extraction**

The dried ginseng powder with 20-mesh sieve was put into the extraction tank. Set parameters: extraction pressure 35MPa, extraction temperature 45℃, extraction time 2.5h, raw material/ entrainer (g/mL) 1:3.0.

Chemical composition analysis methods

The fatty acid analysis were tested by Gas Chromatography-Mass Spectrometer (GC-MS), the contents of sterol were tested by gas chromatography (GC) and the squalene content was determined by High Performance Liquid Chromatography (HPLC).

In vitro whitening test methods



Keratinocytes and melanocytes co-culture model

- The ginseng oil were dissolved in DMSO and added to culture medium at final concentration of 20 ug/mL.
- Same volumen of DMSO was added to the no treated (NT) well as vechile control.
- Arbutin at concentratio of 50 ug/ml was tested as positive control.

- The cells were fixed with 4% formaldehyde for 30 min and then incubated with 0.2% L-DOPA staining solution for 3h at 37℃.

- The melanin staining was observed under microscope, images were taken by automated microscope.

Results & Discussion:

1.Extraction rate of ginseng seed oil

The results show that different extraction methods have a great impact on the extraction rate ranged from 4.0% to 15.0%. High temperature extraction has a higher extraction rate 15.0% and cold pressing is the lowest 4.0%, the difference is nearly three times, suggesting that temperature plays a key role in extraction efficiency.

Table 1 Extraction rate of ginseng seed oils

Extraction conditions	extraction rate %
Cold pressing extraction	**4.0**
N-hexane cold extraction	**9.0**
N-hexane thermal extraction	**15.0**
Ethanol extraction	**5.0**
Supercritical CO_2 extraction	**5.0**

2.Chemical composition analysis of ginseng seed oil

Data showed that ginseng seed oil is a kind of oil with high degree of unsaturation, which mainly contains oleic acid and linoleic acid. The content of oleic acid is very high, ranging from 78.3% to 79.8%, and the content of linoleic acid is 17.3% to18.1%. Compared with the five extraction methods, the influence of the fatty acid composition ratio is smaller.

Table 2 Comparison of fatty acid content in ginseng seed oil with different extraction methods

Extraction conditions	Carbon chain distribution (%)					
	palmitic acid C16:0	Palmitooleic acid C16:1	stearic acid C18:0	oleic acid C18:1	linoleic acid C18:2	linolenic acid C18:3
Cold pressing extraction	**2.0**	**0.2**	**0.3**	**79.8**	**17.3**	**0.4**
N-hexane cold extraction	**1.9**	**0.2**	**0.3**	**79.3**	**17.7**	**0.6**
N-hexane thermal extraction	**1.8**	**0.2**	**0.3**	**79.3**	**18.0**	**0.4**
Ethanol extraction	**2.3**	**0.2**	**0.6**	**78.3**	**18.1**	**0.6**
Supercritical CO_2 extraction	**2.0**	**0.2**	**0.3**	**79.8**	**17.3**	**0.4**

Compared with the five extraction methods, the total sterol content of supercritical CO_2 extraction is the highest at 0.42%, while the lowest cold pressing extraction is only 0.21%, which is half of supercritical CO_2 extraction. The total sterol content of solvent extraction is close, but the squalane extracted by ethanol extraction is higher than other methods, as shown in Table 3.

Table 3 Comparison of sterol in ginseng seed oil with different extraction methods

Extractionconditions(%)	total	stigmasterol	sitosterol	squalene
Cold pressing oil	**0.21**	**0.04**	**0.11**	**0.57**
Cold extraction oil	**0.33**	**0.11**	**0.12**	**0.58**
Thermal extraction oil	**0.34**	**0.11**	**0.12**	**0.59**
Ethanol extraction oil	**0.34**	**0.05**	**0.14**	**1.59**
Supercritical CO_2 extraction oil	**0.42**	**0.07**	**0.18**	**0.71**

3.Whitening effect of ginseng seed oil

Cell staining results showed that when treated with 20 μg/mL of ginseng seed oil, the melanin content was decreased without impact on cell viability in the keratinocyte and melanocyte co-culture cell model.

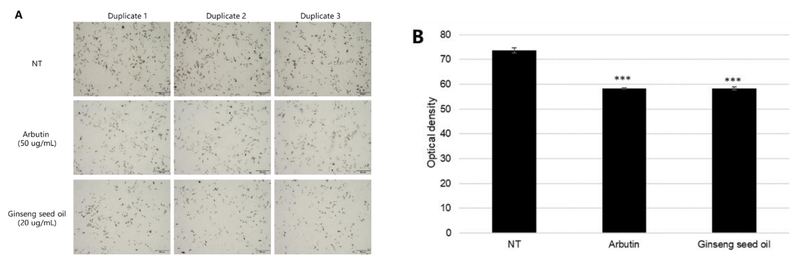


Fig.1 The effect of ginseng seed oil on the melanin contents of melanocyte-keratinocyte co-culture cell system. A: Images of dopa staining; B: Optical density analysis results (n=3), *** p<0.001 *vs* NT group.

Conclusions:

Ginseng seed oil was prepared by different extraction methods, the extraction rate, carbon chain distribution, squalene content were tested, in the meantime, whitening and skin care application were also discussed. The results showed that among the ginseng seed oil obtained by different extraction methods, the extraction rate of cold pressing, both 95% ethanol and supercritical CO_2 was lower, while the extraction rate of using hexane was the highest of 15.0%. The carbon chain distribution between several ginseng seed oil was not significantly different. The seed oil extracted with 95% ethanol had the highest content of squalene of 1.59%. The total sterol and sitosterol content of seed oil extracted by supercritical CO_2 was the highest of 0.34%, 0.18% respectively. The content of stigmasterol in hexane cold extract seed oil was the highest of 0.11%. In the potential application of ginseng seed oil to the skin, 20 μg/mL of oil sample treated the keratinocytes grew well, it was no cytotoxicity, and compared with the blank control, the melanin content was less in melanocytes, similar to the positive control arbutin, which supposed to have whitening effect.

Aknowledgments:

None

References:

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