Original Article

# Characterization of *Arachis hypogaea* L. oil obtained from different extraction techniques and *in vitro* antioxidant potential of supercritical fluid extraction extract


## Abstract

**Aim:** The present investigation was aimed to characterize the fixed oil of *Arachis hypogaea* L. using five different extraction methods: Supercritical fluid extraction (SFE), ultrasound assistance extraction, soxhlet extraction, solvent extraction, and three phase partitioning method. **Materials and Methods:** The SFE conditions (temperature, pressure, and volume of $CO_2$) were optimized prior for better yield. The extracted oils were analyzed and compared for their physiochemical parameters, high-performance thin layer chromatography (HPTLC), gas chromatography-mass spectrometry (GC-MS), and Fourier transform infrared spectrometry (FT-IR) fingerprinting. Anti-oxidant activity was also determined using DPPH and superoxide scavenging method. **Results:** The main fatty acids were oleic, linoleic, palmitic, and stearic acids as obtained by GC-MS. HPTLC analysis revealed the presence of similar major components in chromatograms. Similarly, the pattern of peaks as obtained in FT-IR and GC-MS spectra of same oils by different extraction methods was superimposable. **Conclusion:** Analysis reported that the fixed oil of *A. hypogaea* L. is a good source of unsaturated fatty acid, mainly n-6 and n-9 fatty acid with a significant antioxidant activity of oil obtained from SFE extraction method.



**Key words:**
*Fourier transforms infrared spectroscopy, gas chromatography-mass spectrometry, supercritical fluid extraction*


## Introduction

Peanut (*Arachis hypogaea* L.) is an important oilseed in the world and an important food source of lipids and proteins. Peanut is composed of about 50% lipid and 29% of protein and also contain vitamins and minerals.[1] Peanut oil is one of the major oils in the human diet and rich in unsaturated fatty acids (80%),[2] to which it has attributed its effectiveness in reducing total cholesterol. Because of its beta-sitosterol, it may inhibit cancer growth.[3]

Conventional industrial processing of peanut oil involves pressing and solvent (SOL) extraction. Pressing is less efficient and gives lower yield (40–60%). SOL extraction, although the recovery of oil is in the 90–98% range, has disadvantages including high investment and energy requirements. The hexane used as the most common solvent for extraction of oil is listed among hazardous air pollutants


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**How to cite this article:** Chauhan R, Ahmad I, Khan Y, Tamboli ET, Ahmad S. Characterization of *Arachis hypogaea* L. oil obtained from different extraction techniques and *in vitro* antioxidant potential of supercritical fluid extraction extract. Drug Dev Ther 2016;7:87-91.

associated with neurological and respiratory disorders on prolonged exposure (the International Standard Organization permits only 50 ppm residual hexane in oilseed meal).[4] Hence, there is a need of another technique that is safe, efficient, and less time-consuming. Supercritical fluid extraction (SFE) has emerged as an attractive technique for the extraction of fixed oils. Supercritical $CO_2$ is a greener alternative to the hexane in lipid extraction because it is less toxic and eliminates solvent waste. In addition, energy costs associated with reaching the supercritical state for $CO_2$ have been shown to be less than energy costs associated with conventional solvent distillation.

In this study, we have characterized fixed oil of peanuts extracted by SFE and other conventional methods. Along with it, we also studied the antioxidant potential of extracted fixed oil of peanuts.

## Materials and Methods

### Plant materials

Peanut was collected from a local market and the same was identified and authenticated by a pharmacognosist and voucher specimen was deposited in the Department of Pharmacognosy and Phytochemistry, Jamia Hamdard, New Delhi (Specimen No. BNPL/JH/RCMPH: 05/2012). The peanuts were cleaned, dried, grinded, and passed through a sieve (40 mesh).

### Extraction of fixed oil of peanut

Soxhlet (SOXH) and ultrasound (US)-assisted extraction was done using hexane (ratio 1:3) as a solvent, for 4 h at boiling point in SOXH extraction whereas 1 h at 54 ± 2°C with occasional stirring in US.[5] Fixed oil was obtained by three-phase partitioning (TPP) method as per the reported method.[6] SFE extraction was performed at a pressure of 400 bar and a temperature of 50°C for 75 min at $CO_2$ flow rate of 20 g/min,[7] which was optimized before extraction.

### Physiochemical parameters

Physiochemical parameters (acid value [AV], peroxide value [PV], and iodine value [IV]), ester value, saponification value, and unsaponification value were analyzed according to Indian Pharmacopoeia 2007[8] and matched with reported values.

### High-performance thin layer chromatography profiling

All the five samples obtained through five different extraction procedures (50 times diluted) were applied in duplicate (5.0 µL each) on precoated silica gel 60 $F_{254}$ plates (E. Merck, 0.20 mm thickness) using Linomat V. The chromatograms were scanned at 300 nm after development. The plate was also scanned at 370 nm after spraying with ethanolic sulfuric acid, 10% v/v and drying at 100°C for 5 min in oven.

### Gas chromatography-mass spectrometry analysis of fatty acids

The fixed oils extracted using different extraction methods were analyzed for their fatty acid composition by preparing fatty acid methyl ester.[9]

Agilent 7890A series, gas chromatography-mass spectrometry system was used for analysis attached with CTC-PAL, HP-5 ms automatic sampler, and mass detector. The split less mode at 250°C inlet temperature, 0.1 ml injection volume with helium gas at 1 ml/min, and 70–202°C of oven temperature was used for analysis.

### Fourier transform infrared spectrometry analysis

For the analysis, a Fourier transform-infrared spectrometry spectrophotometer (IRAffinity, Shimadzu) equipped with a deuterated triglycine sulfate detector with a resolution of 4 $cm^{-1}$ was used. The data interval provided by the instrument for a resolution of 4 $cm^{-1}$ is 1.93 $cm^{-1}$. All spectra were recorded from 4000 to 400 $cm^{-1}$. A thin film of the fixed oil was created between two polished KBr disks.[10]

### Assessment of *in vitro* antioxidant activity

Free radical scavenging activity was determined by using DPPH assay previously reported by Hasan *et al*.[11] and Liu *et al*.[12] The method described by Liu *et al*.[13] was used for the determination of anion scavenging activity.

## Results and Discussion

### Evaluation of extraction methods

Table 1 represents the yields of extraction for peanut oil. The result revealed that SFE technique achieved the best result (41.80%) as the extraction was carried out at 400 bars. It was previously reported that oil yield increases with an increase in the pressure that lead to an increase in $CO_2$ density, resulting in a higher solubility and hence higher extraction yield.

### Physiochemical parameter

The physiochemical parameters of the extracted oil of peanut were compared [Table 2]. The extracted oil using SFE technique showed lowest AV (0.52 ± 0.7 mg KOH/g) indicating less free fatty acid. The PV of SFE oil was lowest indicating less rancidity. The IV was highest in TPP showing more unsaturation in extracted oil. The saponification value was lower in SOXH followed by SFE which indicates that the weight of fatty acid was larger in triglycerides.

### High-performance thin layer chromatography profiling

The best separation of constituents of oil samples was found in the solvent system hexane: Diethyl ether: Acetic acid (7:4:1, v/v/v). The comparative high-performance thin layer chromatography fingerprinting of oils extracted was found matching with the presence of different common

compounds at matching $R_f$ [Figure 1]. $R_f$ 0.63 and $R_f$ 0.57 were the major components of all five peanut oil extracted through different methods at 300 nm and 370 nm, respectively. The triglycerides, major components of the fixed oil having high affinity to the solvent system while complex lipids such as phospholipids having low affinity remained at the origin of chromatogram.

**Table 1: Percentage yield of extracted oil of peanut extracted by five extraction methods**

| Extraction procedure | Percentage yield of extracted oil (% w/w) |
|---|---|
| Solvent | 33.68 |
| Soxhlet | 29.88 |
| Ultrasound-assisted | 29.00 |
| Three-phase partitioning | 40.67 |
| Supercritical fluid extraction | 41.80 |

### Gas chromatography-mass spectrometry analysis

The fatty acid compositions of the extracted oil of peanut are shown in Table 3. Oleic acid, linoleic acid, palmitic acid, and stearic acids were found to be the major fatty acids in the five extracted samples. The oil consists of two classes of polyunsaturated fatty acid, n-9 (oleic acid) and n-6 (linoleic acid) at $R_t$ 23.00 min and 22.00 min, respectively [Figure 2]. The oils by SOL extraction showing the highest percent of area are oleic acid and linoleic acid.

### Fourier transform infrared spectrometry fingerprinting of extracted fixed oil of peanut

The intense peaks in the spectra were two intensive bands at 2926 $cm^{-1}$ (aliphatic $CH_2$ asymmetric) and 2856 $cm^{-1}$ (symmetric stretching vibration); at 1741 $cm^{-1}$ is assigned to the C = O stretching vibration of the ester carbonyl functional group of the triglycerides; at 1455 $cm^{-1}$ is observed a band

**Table 2: Physiochemical parameters of the extracted fixed oil of peanut**

| Physiochemical parameter | Extraction method | | | | |
|---|---|---|---|---|---|
| | SOL* | SOXH* | US* | TPP* | SFE* |
| Acid value (mg KOH/g oil) | 0.61±0.8 | 0.59±0.6 | 0.60±0.5 | 0.63±0.5 | 0.52±0.2 |
| Ester value (mg KOH/g Oil) | 302.49±0.7 | 293.62±0.9 | 297.06±0.6 | 301.43±1.2 | 295.42±0.4 |
| Iodine value | 48.03±0.6 | 46.93±1.0 | 47.29±0.8 | 49.16±0.4 | 47.85±0.6 |
| Peroxide value (mEq of active oxygen/kg oil) | 3.38±1.5 | 4.73±0.3 | 4.19±1.3 | 2.58±0.7 | 2.49±0.8 |
| Saponification value (mg KOH/g oil) | 303.10±0.6 | 294.21±0.9 | 297.66±1.0 | 302.06±1.4 | 295.98±1.0 |
| Unsaponification value (g/kg) | 2.16±1.1 | 2.36±0.8 | 2.59±0.6 | 1.63±0.4 | 2.29±0.7 |

*SFE – supercritical fluid extraction; TPP – Three-phase partitioning; SOXH – Soxhlet; US – Ultrasound; SOL – Solvent

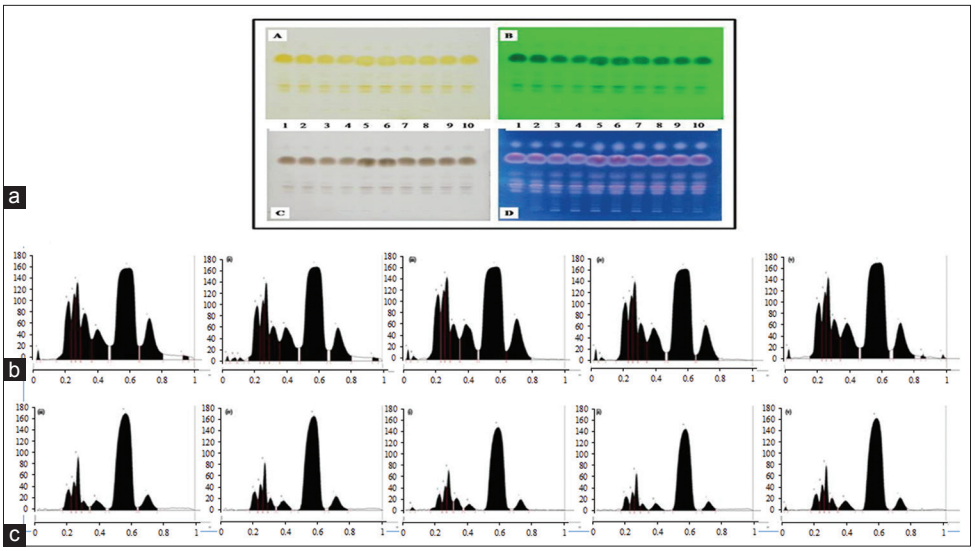


**Figure 1:** (a) High-performance thin layer chromatography fingerprint of peanut oil; derivatization: Lodine vapor A: Day light, B: 254 nm; 10% ethanolic sulfuric acid C: Day light, D: 366 nm; supercritical fluid extraction: Track 1–2, three-phase partitioning: 3–4, soxhlet: 5–6, ultrasound: 7–8, solvent: 9–10; (b) high-performance thin layer chromatography chromatograms (iodine derivatized) at 300 nm; (c) High-performance thin layer chromatography chromatograms (10% ethanoic sulfuric acid derivatized) at 370 nm; (i) supercritical fluid extraction, (ii) three-phase partitioning, (iii) soxhlet, (iv) ultrasound-assisted extraction, (v) solvent extraction

which is assigned to C = H scissors deformation vibration; the bands at 1165 $cm^{-1}$ and 1236 $cm^{-1}$ are assigned to the vibration of the C-O ester groups and $CH_2$ group; the band near 1371 $cm^{-1}$ is assigned to the bending vibration of $CH_2$ groups; at 719 $cm^{-1}$ (overlapping of $CH_2$ rocking), 3007 $cm^{-1}$ (C-H stretching vibration of the cis-double) [Figure 3]. There were some weak peaks at 590 $cm^{-1}$ (-$CH_2$ rocking vibration) and 3474 $cm^{-1}$ (overtone of the glyceride ester carbonyl).

**Table 3: Fixed oil content of fixed oil peanut**

| Fatty acid | Percentage of area | | | | |
|---|---|---|---|---|---|
| | SOL* | SOXH* | US* | TPP* | SFE* |
| Palmitic acid | 12.94 | 11.90 | 12.62 | 12.82 | 12.65 |
| Steric acid | 4.53 | 4.26 | 4.37 | 4.83 | 4.30 |
| Linoleic acid | 32.48 | 29.6 | 31.46 | 30.2 | 32.21 |
| Oleic acid | 43.18 | 40.11 | 42.79 | 40.98 | 40.73 |

*SFE – Supercritical fluid extraction; TPP – Three phase partitioning; SOXH – Soxhlet; US – Ultrasound; SOL – Solvent

### *In vitro* antioxidant activity of supercritical fluid extraction-extracted fixed oil

The peanut oil showed good antioxidant activity, $IC_{50}$ of standard ascorbic acid was 30.09 μg/ml whereas that of oil was 51.17 μg/ml. In superoxide anionic scavenging method,

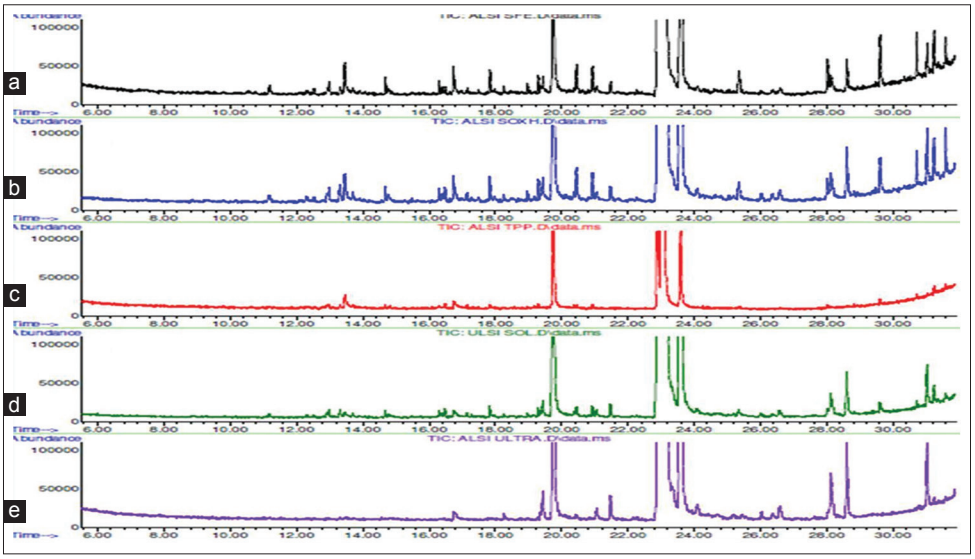


**Figure 2:** Gas chromatography-mass spectrometry chromatograms of fatty acid methyl esters of extracted fixed oil of peanut; (a) supercritical fluid extraction, (b) soxhlet extraction, (c) three-phase partitioning method, (d) solvent extraction, (e) ultrasound-assisted extraction

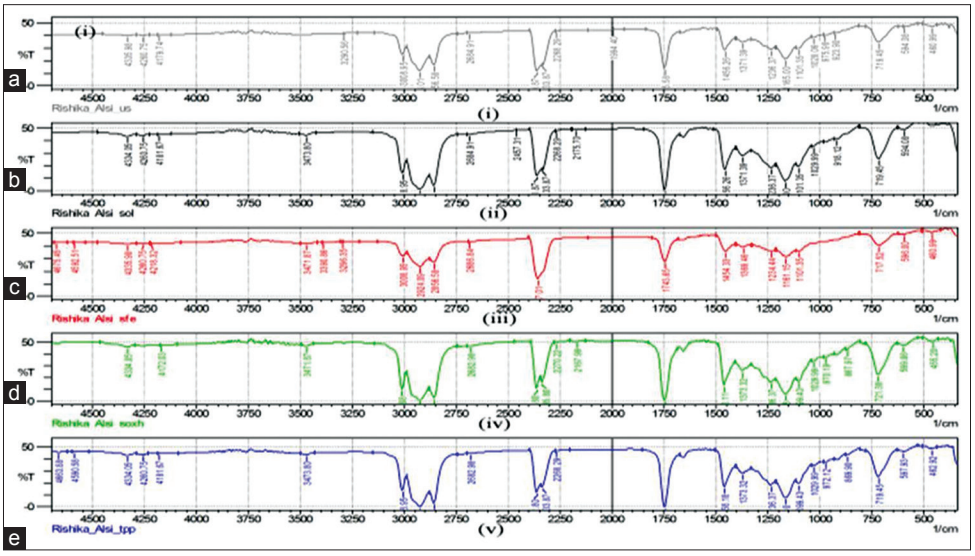


**Figure 3:** Comparative Fourier transform infrared spectrometry spectra of the fixed oil of peanut; (a) ultrasound-assisted extraction, (b) solvent extraction, (c) supercritical fluid extraction, (d) soxhlet extraction, (e) three-phase partitioning

the oil showed $IC_{50}$ of 86.34 μg/ml as compared with standard (ascorbic acid, $IC_{50}$ 217.40 μg/ml). The oxidative stability of peanut oil is highly correlated with the ratio of oleic acid to linoleic acid and as the ratio increases, oxidative stability increases.

## Conclusion

To recover the oil of peanut, different extraction techniques were used. The SFE is a promising technique for the extraction of fixed oil. It has advantages of rapidity, low thermal damage, reduced solvent volume, and the possibility to selectively isolate the components of interest. The fatty acid profile of peanut oil revealed that oleic acid is the main monounsaturated fatty acid. The peanut oil has good antioxidant activity that is related to its lower unsaturation.

**Financial support and sponsorship**
Nil.

**Conflicts of interest**
There are no conflicts of interest.

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