Ultrasonic extraction, composition analysis, in vitro antioxidant and antiproliferative activities of Mango kernel oil from Jinhuang Mango kernel

Chuanjin Wang

Department of Pharmaceutical and Fine Chemicals, School of Chemistry and Chemical Engineering, Nanjing University of Science and Technology, Nanjing, People’s Republic of China

ABSTRACT

Mango kernel oil (MKO) contains a large number of symmetric triglycerides, such as stearic acid, oleic acid and palmitic acid. If MKO is used effectively, it can reduce the waste of resources and environmental pollution. In this study, ultrasonic extraction, composition analysis, in vitro antioxidant and antiproliferative activities of Jinhuang mango kernel oil (JMKO) from Jinhuang mango kernel (JMK) as a product were studied for the first time. The ultrasound extraction yield of JMKO was 15.32%. Forty-three compounds and their relative contents were identified by GC-MS. The total relative content was about 95.71%. JMKO showed moderate antioxidant activities in ABTS assay (0.08 mg/mL as IC$_{50}$ value), DPPH test (IC$_{50}$ value = 0.27 mg/mL). Furthermore, the reducing power of JMKO was dose dependent, and the reducing capacity of the oil was inferior to vitamin C (Vc), which is known to be a strong reducing agent. When concentrations of Vc and JMKO were 11.6 μg/mL and 0.29 mg/mL, their $W_A$ (the total anti-oxidation activity) values were 2.3 and 0.502, respectively. The IC$_{50}$ of JMKO against TE-1 cancer cells, HeLa cancer cells, and MCF-7 cancer cells were (0.43 ± 0.03) mg/mL, (0.83 ± 0.04) mg/mL, and (0.27 ± 0.02) mg/mL, respectively. The results showed JMKO could be employed as natural antioxidants and health care products.

KEYWORDS

Jinhuang Mango kernel oil (JMKO); Chemical constituents; GC-MS; Antioxidant activity; Antiproliferative activity

Introduction

Edible oil from plant origin has continued to receive attention due to their nutritional importance. In particular, oil and fat from plants contained higher contents of unsaturated fatty acids.[1] Mango belongs to the family of Anacardiaceae and has been known as the “king of fruits.” Mango is one of the most important fruits worldwide, with 37–45 million tons produced annually from 2010 to 2014. Although the fruit is cultivated in more than 100 countries, India, China, Thailand, Indonesia, and Mexico are the top five main mango-producing countries, whose annual output account for 62–75% (nearly 28 million tons) of the global yield. In general, mango pulp has been processed into various snacks (e.g., juice, jam, pickle, dried fruit and jelly) in the food industry.[2] However, large amounts of the seeds and their kernels are considered low-value agricultural byproducts or even discarded as waste.[3] There is a lot of scientific evidence about the nutritional profile of mango byproducts. From environmental and food insecurity perspectives, it is extremely important to efficiently utilize mango byproduct, for safer environment and feeding of ever increasing human population.[4] Mango kernel represents about 20% of the whole fruit and 75% of the stone.[5] Mango kernel oil (MKO) may be defined as oil fraction extracted from stone of mango fruit. Mango kernel produces 12–15% edible oil.

CONTACT Chuanjin Wang wangchuanjin@njjust.edu.cn Department of Pharmaceutical and Fine Chemicals, School of Chemistry and Chemical Engineering, Nanjing University of Science and Technology, Nanjing, People’s Republic of China

© 2022 Chuanjin Wang. Published with license by Taylor & Francis Group, LLC. This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.
Studies have disclosed that mango kernel is a potential source of wide range of bioactive compounds and antioxidants.\textsuperscript{[6]} Cardio and hepatic protective effects, anticarcinogenic, anti-aging effects of phenolic compounds are scientifically proven.\textsuperscript{[7,8]} MKO can be utilized for the preservation of fats and oils, supplementation of sunflower oil and tallow with MKO, improved their oxidative stability.\textsuperscript{[9]} Unfortunately, the massive nutritional and commercial potential of MKO is not fully utilized. Chemical characterization of mango kernel revealed that oleic acid is the major fatty acid, followed by palmitic and stearic acids, solid fat index of MKO was zero at human body temperature, the melting point ranges from 32 to 36°C, which offers wide range of applications in trans free options, without partial hydrogenation and as a cocoa butter substitute.\textsuperscript{[10]} On account of abundant mango production, neuteraceutical characteristics, MKO has the potential to become a commercial source of edible oil.

The main methods of extracting MKO from Mango kernel (MK) are solvent reflux method (oil yield: 10.49%), supercritical CO\textsubscript{2} technique (oil yield: 2.5–3.6%), and ultrasonic assisted extraction method (oil yield: 8.01%).\textsuperscript{[11–13]} Jinhuang mango kernel (JMK) is a new mango variety successfully bred by Mr Jinhuang, a fruit farmer in Taiwan. This new variety is the result of crossing of male Keitt variety and female White variety. It is now widely grown in mainland of China. Jinhuang mango (JM) has strong resistance to adversity, good yield, large fruit, good appearance, late ripening, storage and transportation, sweet and juicy taste, Less fiber, good quality.\textsuperscript{[14]}

In this study, the ultrasonic extraction process of JMKO was determined. Furthermore, the constituents of JMKO were identified by GC-MS. Besides, we also tested the antioxidant activity of JMKO compared with Vc on free radical scavenging and its inhibitory rates compared with cisplatin (DDP) on three types of human tumor cells proliferation (TE-1 cancer cells, HeLa cancer cells, and MCF-7 cancer cells). The results offer a theoretical reference for efficient extraction of JMKO from JM by ultrasonic-assisted extraction. JMKO obtained exhibit significant antioxidant and antiproliferative activities. JMKO can be used as a natural source of healthy food ingredients. The research results could improve the sufficient utilization of JM resources.

Materials and methods

Chemicals

Methyl alcohol (CH\textsubscript{3}OH), ethyl alcohol (C\textsubscript{2}H\textsubscript{5}OH), potassium ferricyanide (K\textsubscript{3}Fe(CN)\textsubscript{6}), trichloroacetic acid (CCl\textsubscript{3}COOH), ferric chloride hexahydrate (FeCl\textsubscript{3}·6H\textsubscript{2}O), sodium hydrogen phosphate (Na\textsubscript{2}HPO\textsubscript{4}·12H\textsubscript{2}O), sodium dihydrogen phosphate (NaH\textsubscript{2}PO\textsubscript{4}·2H\textsubscript{2}O), sodium sulfate (Na\textsubscript{2}SO\textsubscript{4}), potassium persulfate (K\textsubscript{2}S\textsubscript{2}O\textsubscript{8}), 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2'-Azinobis (3-ethylbenzothiazoline-6-sulfonic acid ammonium salt (ABTS), and vitamin C (Vc) were obtained from Nanjing Jiaoziteng Scientific Equipment Co., Ltd (Nanjing, China). All of the above chemical reagents are at least analytical level. Cisplatin (DDP), Dimethyl sulfoxide (DMSO, cell-culture grade), and chromatographic methanol were provided from Sigma Chemical Co., Ltd (Shanghai, China). Polyamide (Particle size: 0.125 mm-0.150 mm) was from Jiangsu Changfeng Chemical Co., Ltd (Changzhou, China). Powdered activated carbon (Particle size: 1.0 μm-150 μm) was from Shanghai Bilang Environmental Protection Technology Co., Ltd (Shanghai, China).

Extraction and gas chromatography/mass spectrometry (GC/MS) analyses of JMKO

Ultrasonic extraction method was selected to extract JKMO according to the previous research results.\textsuperscript{[15]} In briefly, JMK (Yulin, Guangxi Province, China) was crushed, passed through 20 mesh sieve, then soaked in petroleum ether [60°C–90°C, 1:30 (g/mL)] for 3 h at room temperature, extracted by ultrasonic for 30 min at 60°C and the extraction was repeated 3 times. The product was obtained after removing residue by filtration and evaporating petroleum ether using rotary evaporation. The oil was dried over anhydrous Na\textsubscript{2}SO\textsubscript{4}. weighted. The color of dried oil was brown and yellow. The oil was then weighed and stored at –18°C until further analysis.
The chemical composition of JMKO was investigated by GC-MS.\textsuperscript{[16]} GC-MS analysis was performed using a gas chromatograph/mass spectrometry equipped with a fused silica capillary column (HP-5 MS, 0.250 mm × 30 m, film thickness 0.25 μm), a solid phase microextraction needle, and coupled with a mass spectrometer detector recording at 70 eV (Trace1310 ISQ, Thermo Fisher Scientific, Massachusetts, USA). Full-scan spectra were acquired (5 scans s\textsuperscript{-1}) over the range m/z 40–600. Sample pretreatment method: Weighed 3 g of the sample into a 10 mL headspace bottle with water bath at 80°C for 30 min, and then extracted it with a solid phase microextraction needle for 30 min. After the extraction, the extraction needle was desorbed at the inlet for 5 min. GC/MS was performed under the following conditions: The carrier gas is helium (1.6 mL/min). The injector, source, and quadrupole temperatures are set at 230°C, 230°C, and 150°C. The initial temperature of oven was 60°C for 5 min, then increased to 120°C at a rate of 4°C/min for 5 min, increased to 170°C at a rate of 3°C/min for 2 min, and increased to 280°C at a rate of 10°C/min for 5 min. split ratio was splitless. The collected mass spectra were retrieved by using NIST spectrum library to identify the volatile components in the samples, and the relative contents of each component were analyzed by area normalization method.

**In vitro antioxidant potential of JMKO**

Vc is a frequently used antioxidant. Therefore, it was used as positive substance to evaluate the anti-oxidation activity of JMKO in this study. The anti-oxidation activity of JMKO was tested by three methods, including DPPH free radical scavenging, ABTS free radical scavenging, and ferric reducing power tests with UV-visible spectrophotometer of N4S type (Shanghai Yidian Analytical Instrument Co., Ltd. China). JMKO was dissolved in ethanol to formulate the desired final concentrations. All the tests were repeated three times, and the average values were calculated.

**DPPH free radical scavenging activity assay**

The scavenging activity of DPPH to free radical was tested in the light of the method described by Ting et al., making a few modifications.\textsuperscript{[17,18]} The specific operation steps were as follows: 3 mL of DPPH solution (1 × 10\textsuperscript{-4} mol/mL) was added to 3 mL various of concentrations (0.1, 0.2, 0.5, 0.7, 0.9, 1.1, 2.0, 4.0, and 8.0 mg/mL) of JMKO solution in sequence. The mixture solution was placed for 30 min in the dark at room temperature, and then its absorbance was determined at 517 nm with spectrophotometer. Ethanol was used as reference. Concentrations (0.34, 1.36, 2.34, 3.38, 3.40, 5.10, 6.80, and 10.20 μg/mL) of Vc were set in this test as a positive control. The absorbance value was recorded as A. 3 mL of sample solution and 3 mL of ethanol were blended and measured at 517 nm with spectrophotometer. The absorbance value was recorded as A₀. 3 mL of 1 × 10\textsuperscript{-4} mol/mL DPPH and 3 mL of ethanol were blended and measured at 517 nm with spectrophotometer. The absorbance value was recorded as A₁. Antioxidant effects were expressed using radical scavenging rate. The calculation formula of radical scavenging rate is as follows:

\[
\text{Radical scavenging rate} = \left[1 - \left(\frac{A}{A_0}\right)\right] \times 100\%
\]

**ABTS free radical scavenging activity assay**

The radical scavenging activity of JMKO against radical cation (ABTS\textsuperscript{+}) was determined according to previous related research report of Zeng et al.\textsuperscript{[19]} with a few modifications. ABTS\textsuperscript{+} was generated by reaction ABTS\textsuperscript{+} solution (7 mmol/L, 5 mL) and K\textsubscript{2}S\textsubscript{2}O\textsubscript{8} solution (14 mmol/L, 88 μL), and the reaction product would be kept out of the light at room temperature for one day. Then, ABTS\textsuperscript{+} solution was diluted with C\textsubscript{2}H\textsubscript{5}OH to an absorbance of 0.68–0.72 at 734 nm. In short, 1 mL of ABTS\textsuperscript{+} dilute solution was mixed with 5 mL series of concentrations (10.2, 20.4, 40.8, 61.2, 81.6, and 102.0 μg/mL) of JMKO in sequence and mixed. Concentrations (0.52, 1.39, 2.43, 3.47, 5.20, and 6.93 μg/mL) of Vc were set in this test as a positive control. After reaction at room temperature for 0.5 h in dark condition, the
absorbance was determined at 734 nm. The absorbance value was recorded as A. Ethanol was used as reference. 1 mL of ABTS⁺ dilute solution and 5 mL of ethanol were blended, placed for 0.5 h in the dark, and determined at 734 nm with UV spectrophotometer. The absorbance value was recorded as $A_0$. Antiproliferative effects were expressed using radical scavenging rate. The calculation formula of radical scavenging rate is as follows:

$$\text{Radical scavenging rate} = \left[\frac{(A_0 - A)}{A_0}\right] \times 100\%$$

**Ferric reducing antioxidant power assay**

The reducing power of JMKO was examined according to previous related research report with a few modifications. In short, 1 mL of sample solution (0.03, 0.06, 0.10, 0.18, 0.23 and 0.29 mg/mL) JMKO) was mixed with 2.5 mL of PBS (pH 7.5) and 2.5 mL of 1% K$_3$Fe(CN)$_6$. The mixed solution was incubated in 50°C water bath for 30 min. Then, 2.5 mL of CCl$_3$COOH solution (10%) was added into previous the mixed solution, and mixed well. Then, the mixture was centrifuged at 6000 rpm for 30 min. About 2.5 mL of the upper layer was mixed with 2.5 mL of H$_2$O, 0.5 mL of aqueous solution of FeCl$_3$ (1%). After 10 min, at 700 nm, the absorbance value (A) was determined using a UV spectrophotometer. The reference absorbance value ($A_0$) was detected from H$_2$O. Concentrations (2.3, 4.6, 6.9, 9.2, and 11.6 μg/mL) of Vc were set in this test as a positive control. The total anti-oxidation activity was expressed as $W_A$.

$$W_A = A - A_0$$

**Antiproliferative effect of JMKO in vitro**

Cell counting kit-8 (CCK-8), 96-well culture plates, tissue culture flask, dulbecco’s modified eagle’s medium (DMEM), 100 mg/mL streptomycin and 100 IU/mL penicillin, trypsin-EDTA digestive fluid, TE-1 cancer cells, Hela cancer cells, MCF-7 cancer cells were supplied by Jiangsu KeyGEN BioTECH Co., Ltd (Nanjing, China). The antitumor cell growth of JMKO in vitro was detected using the CCK-8 assay, as described previously in living cells (TE-1, HeLa, MCF-7). TE-1, HeLa, MCF-7 cells were cultured in DMEM supplemented with 4.5 mg/mL glucose, 10% FCS, 100 U/mL penicillin G, 2 mM glutamine, and 100 mg/mL streptomycin. In short, cancer cells were counted, transferred into 96 well microtiter plates, and incubated using a XD-101 type incubator (Matsushita Electric Industrial Co., Ltd. Menmakami, Japan) for 24 h prior to the addition of JMKO. JMKO was dissolved in DMSO, and diluted in sterile media, to obtain the appropriate concentration. Exponentially growing cells of TE-1, HeLa, MCF-7 was made into single cell suspensions with 0.25% trypsin, at a cell concentration of 3.5 × 10⁴/mL, 3.5 × 10⁴/mL and 3.5 × 10⁴/mL, respectively. About 100 μL cells were seeded into each well of a 96-well plate. TE-1, HeLa, MCF-7 cells were incubated for 24 h before they were treated with JKMO which were in a medium containing 0.1% DMSO, which showed no inhibitory effect on cell growth. This study was carried out using six different drug concentrations (0.02, 0.06, 0.19, 0.56, 1.67, and 5.00 mg/mL). Each well was added 10 μL of the appropriate drug. DDP (10 μg/mL) was set in this test as a positive control. Control cells were treated with the same volume of serum-free RPMI 1640 including 0.1% DMSO. The volume of DMSO added to each well was only 100 μL. After cells had been maintained for 72 h, 10 μL CCK-8 was added to each well. One hour later, the cell viability ratio was detected from the absorbance measured at 450 nm using enzyme standard instrument (EL-x800 type, American Berten Instrument Co., Ltd. Vermont, USA). Each sample was assayed in triplicate, and each assay was repeated twice. Results are expressed as the concentration yielding 50% inhibition (IC$_{50}$).

The inhibition rate(%)=$\left[\frac{(A_{\text{control}} - A_{\text{experiment}})}{A_{\text{control}}}\right] \times 100\%$
Data were expressed as mean ± SD. One-way analysis of variances and Fisher’s least significant difference were carried out using SAS 8.13. Differences were significant at P*< 0.05.

Statistical analysis

Data are presented as mean ± standard deviation. Statistical analysis was performed with a One-way ANOVA with significance taken to be p < .05 using Minitab 17 Statistical Software (Minitab Inc., State College, PA) to detect statistically significant differences between means. A Tukey’s multiple comparison test was used to determine which of the means are statistically different.

Results and discussion

GC-MS of JKMO

The extraction yield of JKMO was 15.32%. GC-MS was used for identification of compounds. The results of the GC-MS of JKMO showed 43 components listed in Tables 1 and 2, 2-dimethyl-3-propyl-Oxirane, 5-methyl-2-Furancarboxaldehyde, 1, 3-Octanediol, 1-Octen-3-ol, 1-(1H-pyrrol-2-yl)-Ethanone, 6-Propyl-5,6-dihydro-2H-pyran-2-one, Anethole, 3-Methyl-2-(2-pentenyl)-2-cyclopenten-1-one, 4-Hexyl-2,5-dihydro-2, 5-dioxo-3-furancetic acid, and 3,4-Dihydro-8-hydroxy-3-methyl-1H-2-benzopyran-1-one were identified as the main components by GC-MS. Their relative contents were 4.96%, 4.75%, 4.16%, 5.23%, 6.41%, 12.62%, 7.52%, 4.29%, 7.30%, 8.71%, respectively.

Ri is obtained by the calculation of experiment; Ri* is obtained by http://webbook.nist.gov/chemistry, NIST14s. lib or www.webbook.nist.gov; “.” represents no or not detected.

The results indicated that the yield of JKMO (15.32%) under ultrasound assisted extraction was significantly higher than previous research (10.49%, 2.5–3.6%, 8.0%).[11–13] 43 components were identified through GC-MS analysis method. The nonpolar PLE (integrating green pressurized-liquid extraction) extract obtained from mango seed kernel was analyzed by GC-q-TOF-MS to characterize seven lipidic components containing citric acid, palmitic acid, oleic acid, stearic acid, linoleic acid, eicosapentaenoic acid and β-sitosterolas.[22] The large increase in extraction rate and types of could be mainly due to thermal effects and cavitation effects of the ultrasound wave, which cause disruption of the cell wall and increase of mass transfer of oil from JKM.

Evaluation of JKMO antioxidant activity in vitro

DPPH free radical scavenging capacity of JKMO: The research findings of DPPH free radical scavenging activity of JKMO are shown in Figure 1 and compared with Vc as a positive control (Figure 2). DPPH radical, can accept a hydrogen radical or an electron to become a stable molecule and make its absorbance reduction at 517 nm, which has been usually used to detect the radical scavenging activity of some compounds. The higher the DPPH radical scavenging values, the higher the antioxidant activity. Figure 1 shows that radical scavenging rate to DPPH increased from 35.5% to 98.5%, when the concentration of JKMO added from 0.1 to 8.0 mg/mL. The DPPH free radical scavenging capacity of JKMO was improved when the concentration of JKMO was enhanced (Figure 1). The DPPH radical scavenging rate of Vc increased from 30.4% to 98.6%, when the concentration of Vc added from 0.34 to 10.00 µg/mL (Figure 2). The DPPH scavenging activity of JKMO was found to be significantly weaker than that of Vc. The antioxidant activity of the extract was expressed as IC50. The IC50 value was defined as the concentration (in mg/mL) of extracts that scavenged the DPPH radical by 50%. The IC50 values could be calculated from plotted graph of percentage scavenging activity against the concentration of JKMO. The IC50 value of JKMO was 0.27 mg/mL. The research findings showed that JKMO contain compounds could donate electron/hydrogen easily and possessed a potential
Table 1. Identified compounds of JMKO by GC-MS analysis.

| No | Retention/Content | Compounds Name | Molecular formula | Rf(Ri)* |
|----|-------------------|-----------------|-------------------|---------|
| 1  | 5.73 4.96         | 2,2-dimethyl-3-propyl-Oxirane | C_{12}H_{18}O | 742 (743) |
| 2  | 6.33 2.19         | 2,2-dimethyl-pentanal | C_{12}H_{20}O | 824 (821) |
| 3  | 7.57 4.72         | 5-methyl-2-Furancarboxaldehyde | C_{11}H_{12}O_2 | 967 (966) |
| 4  | 9.43 4.16         | 1,3-Octanediol | C_{12}H_{22}O_2 | 974 ( ) |
| 5  | 9.49 5.23         | 1-Octan-3-ol | C_{11}H_{22}O_2 | 988 (986) |
| 6  | 10.16 0.89        | D-Limonene | C_{10}H_{16} | 1031 (1032) |
| 7  | 10.87 2.27        | 6-hydroxy-2-Hexanone | C_{10}H_{20}O_2 | 1054 ( ) |
| 8  | 11.49 6.41        | 1-(1-Hydrop-2-yl)-ethanone | C_{10}H_{18}NO | 1063 (1063) |
| 9  | 12.32 0.62        | 2-Amino-3-(4-hydroxyphenyl)-propanoic acid | C_{11}H_{15}NO_2 | 1076 ( ) |
| 10 | 12.66 0.61        | 2-methoxy-phenol | C_{12}H_{14}O_2 | 1086 (1086) |
| 11 | 12.82 0.77        | 4,4,8-Trimethyl-7-none-2-one | C_{13}H_{20}O_2 | 1092 ( ) |
| 12 | 13.32 0.75        | Nonanal | C_{10}H_{18}O | 1101 (1102) |
| 13 | 14.1 0.78         | 1-methyl-1H-Pyrole-2-carboxaldehyde | C_{11}H_{18}O_3 | 1146 ( ) |
| 14 | 15.9 1.12         | Benzoic acid | C_{7}H_{6}O_2 | 1179 (1178) |
| 15 | 16.43 3.38        | Naphthalene | C_{10}H_{8} | 1188 (1190) |
| 16 | 16.98 1.30        | Menthol salicylate | C_{10}H_{12}O_3 | ( ) |
| 17 | 19.68 0.69        | Benzoic Acid, TMS derivative | C_{10}H_{16}O_2Si | 1249 (1250) |
| 18 | 19.94 0.55        | 4-methoxy-benzaldehyde | C_{10}H_{16}O_2 | 1267 (1270) |
| 19 | 20.66 12.62       | 6-Propyl-5,6-dihydro-2H-pyran-2-one | C_{11}H_{16}O_2 | 1268 (1267) |
| 20 | 21.11 1.48        | α-ethyliden-benzeneacetaldelye | C_{10}H_{18}O_2 | 1275 (1274) |
| 21 | 21.94 7.52        | Anethole | C_{10}H_{12}O_3 | 1287 (1286) |
| 22 | 22.15 0.76        | 1-methyl-naphthalene | C_{11}H_{14}O | 1301 (1299) |
| 23 | 23.45 0.45        | 1,3,3-Trimethoxybenzene | C_{12}H_{12}O_3 | 1317 (1317) |
| 24 | 24.3 1.39         | 2-Furancarboxyllic acid, 1-ethylundecyl ester | C_{18}H_{20}O_2 | 1336 ( ) |
| 25 | 24.49 1.86        | 2,2,6,7-Tetramethyl-10-oxatricyclo[4.3.0.1(7)1decan-5-one | C_{18}H_{22}O_2 | 1348 ( ) |
| 26 | 24.8 0.77         | 2,6-dimethoxy-phenol | C_{8}H_{10}O_3 | 1368 (1367) |
| 27 | 25.6 1.19         | Copaeone | C_{10}H_{16} | 1378 (1376) |
| 28 | 26.13 0.73        | 1-tetradecane | C_{14}H_{30} | 1391 (1391) |
| 29 | 26.27 4.29        | 3-Methyl-2-(2-pentenyl)-2-cyclopentan-1-one | C_{11}H_{18}O | 1395 (1396) |
| 30 | 26.36 0.63        | Tetradecane | C_{14}H_{30} | 1408 ( ) |
| 31 | 26.65 0.80        | α-furfurylidene-α-furylmethyamine | C_{10}H_{12}NO_2 | 1416 ( ) |
| 32 | 26.83 1.27        | Caryophyllene | C_{12}H_{18} | 1419 (1417) |
| 33 | 26.91 0.52        | 1-tyrophanamide | C_{12}H_{12}ClO_2 | 1424 ( ) |
| 34 | 27.15 0.62        | (1aR,7 R,7aR,7bS)-1a,2,3,5,6,7,7a,7b-Octahydro-1,1,7,7a-tetramethyl-1H-cyclopenta(a)napththalene | C_{15}H_{24} | 1432 (1434) |
| 35 | 27.67 0.60        | Humulene | C_{15}H_{26} | 1452 (1452) |
| 36 | 28.36 7.30        | 4-Hexyl-2,5-dihydro-2,5-dioxo-3-furanacetic acid | C_{12}H_{20}O_3 | 1482 ( ) |
| 37 | 28.61 0.75        | (2 R,4aR,8aR)-1,2,3,4,4a,5,6,8-acid8-Octahydro-4a,8-dimethyl-2-(1-methylthienyl) naphthalene | C_{12}H_{24} | 1495 (1494) |
| 38 | 29.18 0.51        | 1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylthyl)-, (15-cis)-Naphthalene | C_{15}H_{24} | 1519 (1519) |
| 39 | 29.55 8.71        | 3,4-Dihydro-2-hydroxy-3-methyl-1H-2-benzopyran-1-one | C_{10}H_{10}O_3 | 1639 ( ) |
| 40 | 32.76 2.03        | 1-(cyclohex-3-ethyl)-cyclohexane | C_{15}H_{26} | 1742 ( ) |
| 41 | 34.03 0.55        | Ficusin | C_{11}H_{18}O_3 | 1849 (1847) |
| 42 | 36.63 0.47        | Hexadecanoic acid, ethyl ester | C_{12}H_{22}O_2 | 1997 (1996) |
| 43 | 37.81 0.75        | Palmitic Acid, TMS derivative | C_{19}H_{36}O_2Si | 2053 (2054) |

DPPH radical scavenging capacity. Anethole was identified as the main component by GC-MS. Its relative content was 7.52%. It has been previously reported that anethole has the strongest scavenging capacity against DPPH radicals.\(^{[23]}\)

Scavenging effect on ABTS radical of JMKO: This method is based on the ability of antioxidants to quench the long-lived ABTS radical cation, a blue/green chromophore with characteristic absorption at 734 nm. The result of scavenging effect on ABTS free radical of JMKO was displayed in Figure 3 and compared with ascorbic acid (Vc) as a positive control (Figure 4). Figure 3 shows that ABTS free radical scavenging rate improved from 0.0% to 64.5%, when the concentration of JMKO enhanced from 10.2 to 102.0 μg/mL. Free radical scavenging ability of
JMKO positively associated with concentration growth. The ABTS radical scavenging rate of Vc increased from 48.1% to 99.5%, when the concentration of Vc added from 0.52 to 6.93 μg/mL (Figure 4). Although the scavenging effect on ABTS free radical of JMKO was lower than that of Vc, it still reached 64.5% at the concentration of 102 μg/mL. The antioxidant activity of the extract was expressed as IC_{50}. The IC_{50} value was defined as the concentration (in mg/mL) of extracts that scavenged the ABTS radical by 50%. The IC_{50} values could be calculated from the plotted graph of percentage scavenging activity against the concentration of JKMO. The IC_{50} value of JMKO was 0.08 mg/mL. The research findings exhibited that JMKO possessed a good ABTS free radical scavenging ability. Anethole contained in the oil was also reported to have the strongest scavenging capacity against ABTS cation radicals.[23]

Reducing power of JMKO: The reducing power of a substance, which could act as an important indicator of its potential anti-oxidation capacity, was detected using a slightly modified Fe^{3+}-to-Fe^{2+} reducibility test. In this study, the yellow of the test solution changed to a variety of shades of blue and
green depending on the reducing activity of compounds and extracts. The presence of reducing agents in the test solution caused the reduction of the Fe³⁺/ferricyanide complex to Fe²⁺ form and the Fe²⁺ can be detected by monitoring the formation of Perl’s Prussian blue at 700 nm using an ultraviolet spectrophotometer.

As a matter of fact, a greater absorbance related to a higher reducing activity. The results in Figure 5 exhibits that the reducing activity was improved as the concentration of JMKO was raised. The reducing ability of JMKO was lower than that of Vc (Figure 6). Although the reducing power of JKMO was lower than that of Vc, it still reached 0.502 at the concentration of 0.29 mg/mL. The results revealed that JMKO could act as electronic donors and reacted with free radicals to make them to more stable products and therefore terminated radical chain reactions.
Antiproliferative effect of JMKO in vitro

To estimate the anti-tumor activity of JMKO, cytotoxicity studies in vitro were also performed on TE-1, HeLa, and MCF-7 cancer cells using CCK-8 experimental method, as shown in Figure 7 and Table 2. The data presented are mean ± standard deviation of triplicate samples and the difference was considered significant at \(p^*<0.05\). The research findings showed that JMKO exhibited significant growth inhibitory to TE-1, HeLa, and MCF-7 cancer cells according to a concentration-dependent manner. The IC\(_{50}\) values of JMKO against TE-1, HeLa, and MCF-7 cells were (0.43 ± 0.03) mg/mL, (0.83 ± 0.04) mg/mL, and (0.27 ± 0.02) mg/mL, respectively. JMKO exhibited a higher inhibition on the growth rate of MCF-7 cancer cells. The inhibition rates of DDP (10 μg/mL) to TE-1, HeLa, and MCF-7 tumor cells were 92.5%, 92.5% and 94.5%, respectively. The experimental results showed that the inhibition rates of JKMO on TE-1, HeLa, and MCF-7 tumor cells were significantly lower than that of DDP. The results indicated that JKMO only could be used as a health food, but not as an anti-tumor drug. Studies demonstrated that anethole, 3-Methyl-2-(2-pentenyl)-2-cyclopenten-1-one and

![Figure 5](image1.png)

**Figure 5.** Reducing power of JMKO.

![Figure 6](image2.png)

**Figure 6.** Reducing power of Vc.
3,4-Dihydro-8-hydroxy-3-methyl-1H-2-benzopyran-1-one major components in JKMO was playing a dominant role in anti-cancer activity.\textsuperscript{[24–26]} In addition, coumarin compounds also have good antioxidant properties.\textsuperscript{[27]} Studies have shown that antioxidants may slow or possibly counteract cancer development and progress.\textsuperscript{[28]} In this research, the observed anticancer activity of JKMO might be linked to its ability to neutralize free radicals in these cancer cells. The antiproliferative activity observed for JKMO could not be attributed to a single component but to the possible synergistic effect of some of the compounds present in the oil.

**Conclusion**

Plant oil is a natural plant extract, which has anti-bacterial, anti-cancer, anti-inflammatory, antioxidant, and growth-promoting effects.\textsuperscript{[29]} In this study, JKMO was successfully extracted from JMK by ultrasonic extraction. In briefly, JMK was crushed, passed through 20 mesh sieve, then soaked in petroleum ether [60°C~90°C, 1:30 (g/mL)] for 3 h at room temperature, and extracted by ultrasonic for 30 min at 60°C. Under these conditions, the extraction yield of JKMO is 15.32% which is higher than previous literature reports.\textsuperscript{[11–13]} The chemical constituents of JKMO were separated and identified by GC-MS. A total of 43 compounds were identified. In this article, we also tested the antioxidant activity of JKMO on free radical scavenging for the first time. JKMO has good effect of scavenging on DPPH. The IC\textsubscript{50} value of JKMO was 0.27 mg/mL. JKMO has obviously good effect of scavenging on ABTS. The IC\textsubscript{50} value of JKMO was 0.08 mg/mL. JKMO also had remarkable, dose-dependent reducing ability to iron. When the concentration of JKMO was 0.29 mg/mL, its reducing power still reached 0.502. The cell proliferation inhibition assay (CCK-8 method) was used to determine the inhibitory effects of JKMO on TE-1, HeLa, and MCF-7 cancer cells \textit{in vitro} for the
first time. The IC₅₀ values of JMKO against TE-1, HeLa, and MCF-7 cancer cells were (0.43 ± 0.03) mg/mL, (0.83 ± 0.04) mg/mL, and (0.27 ± 0.02) mg/mL, respectively. JMKO exhibited a higher inhibition on the growth rate of MCF-7 cancer cells. In conclusion, JMKO extracted from JMK has high application value in the production of food and health products due to good antioxidant capacity and antitumor effect. It can well avoid the waste of JKM resources.

Acknowledgments

This work was supported by pharmaceutical engineering undergraduate graduation design project of Nanjing University of Science and Technology. The author thanks Song Lv, Jiangsu Keygen Biotech Co., Ltd, for the help with anti-tumor assay in vitro. The author appreciates Xuejin Yu, Qingdao Sci-tech Innovation Quality Testing Co., Ltd, for the help with GC-MS analysis. This article does not contain any studies with human or animal subjects. The authors declare that they have no conflict of interest.

Disclosure statement

No potential conflict of interest was reported by the author(s).

References

[1] Tan, C. P.; Man, Y. B. Differential Scanning Calorimetric Analysis of Edible Oils: Comparison of Thermal Properties and Chemical Composition. J. Am. Chem. Soc. 2000, 77, 143–155. DOI: 10.1021/ja002796o.

[2] Bhardwaj, R. L.; Pandey, S. Juice blends-A Way of Utilization of Under-utilized Fruits, Vegetables, and Spices: A Review. Crit. Rev. Food. Sci. Nutr. 2011, 51, 563–570. DOI: 10.1080/10408391003710654.

[3] Torres-Leon, C. R.; Rojas, J. C.; Contreras-Esuquiel, L.; Serna-Cock, R. E.; Belmares, C.; Aguilar, C. N. Mango Seed: Functional and Nutritional Properties. Trends. Food. Sci. Technol. 2016, 55, 109–117. DOI: 10.1016/j.tifs.2016.06.009.

[4] Muhammad, N.; Muhammad, I.; Anjum, K. Promising Features of Mango (Mangifera Indica L.) Kernel Oil: A Review. J. Food. Sci. Technol. 2016, 53, 2185–2195. DOI: 10.1007/s13197-015-2166-8.

[5] Ahmed, E. M.; Saied, M. D.; Eman, H. E.; Reham, M. E. Egyptian Mango By-product Compositional Quality of Mango Seed Kernel. Food. Chem. 2007, 103, 1134–1140. DOI: 10.1016/j.foodchem.2006.10.017.

[6] Jafari, M.; Gharachorloo, M.; Hemmaci, A. H. The Stabilizing Effect of Three Varieties of Crude Mango Seed Kernel Oil on Tallow. J. Food. Biosci. Technol. 2014, 4, 31–36.

[7] Mohdaly, A. A.; Smetanska, A. I.; Ramadan, M. F.; Sarhan, M. A.; Mahmoud, A. Antioxidant Potential of Sesame (Sesamum Indicum) Cake Extract in Stabilization of Sunflower and Soybean Oils. Ind. Crops. Prod. 2011, 34, 952–959. DOI: 10.1016/j.indcrop.2011.02.018.

[8] Diego, B. V.; Gerardo, A. R.; Andres, F. G. O.; Sandra, J. M.; Andrea, P.; Alejandro, C.; Fabián, P. A.; Elena, I. Supercritical Antisolvent Fractionation as a Tool for Enhancing Antiproliferative Activity of Mango Seed Kernel Extracts against Colon Cancer Cells. J. Supercrit. Fluid. 2019, 152, 1–11. DOI: 10.1016/j.supflu.2019.104563.

[9] Abdalla, E. M.; Darwish, S. M.; Ayad, E. H. E.; El-hamahmy, R. M. Egyptian Mango By-product: Antioxidant and Antimicrobial Activities of Extract and Oil from Mango Seed Kernel. Food. Chem. 2007, 103, 1141–1152. DOI: 10.1016/j.foodchem.2006.10.026.

[10] Kittiphoon, S.; Sutasinee, S. Mango Seed Kernel Oil and Its Physicochemical Properties. Int. Food. Res. J. 2013, 20, 1145–1149. DOI: 10.11111/joim.12123.

[11] Gu, Y. P.; Yan, Z. Y.; Huang, S. Q.; Pang, Y. F.; Duan, Z. H.; Luo, Y. H. Study on the Optimization of Extraction Technology of Mango Kernel Oil by Response Surface Methodology. Food. Ind. 2018, 39, 124–128.

[12] Olugbenga, O. A.; Balaraman, M. Qualitative and Quantitative Characterization of Mango Kernel Oil Extracted Using Supercritical CO₂ and Solvent Extraction Techniques. Helion. 2019. DOI: 10.1016/j.helion.2019.e03068.

[13] Wu, X.; Jia, Y. Q.; Li, Z.; Mao, P. Study on Ultrasonic Assisted Extraction of Mango Kernel Oil. Cereal. Food. Ind. 2020, 27, 30–35.

[14] Tan, D. J.; Wang, W. L.; Zheng, S. F.; Huang, X. Y.; Tang, X. H. Introducing Trials of 5 Mango Varieties in Longzhou, Guangxi. Chin. Fruits. 2014, 2, 46–48.

[15] Gabriele, C.; Anna, R.; Eduardo, M. S.; Michele, D. L.; Emilia, L. B.; Luca, F.; Matteo, B.; Pietro, C.; Anna, R. C.; Francesca, A. Ultrasound-Assisted Extraction, Chemical Characterization, and Impact on Cell Viability of Food Wastes Derived from Southern Italy Autochthonous Citrus Fruits. Antioxid. 2022, 11, 1–12. DOI: 10.3390/antiox.11020285.
[16] Feng, X.; Jiang, Z. T.; Wang, Y. Composition Comparison of Essential Oils Extracted by Hydrodistillation and Microwave-assisted Hydrodistillation from Amomum Tsao-ko in China. J. Essent. Oil. Bear. Pl. 2010, 3, 286–291. DOI: 10.1080/0972060X.2010.10643823.

[17] Du, B.; Ge, Y. H.; Li, C. Y.; Li, X.; Wei, M. L.; Tang, Q. Extraction and Antioxidant Activity in Vitro of Ginger Essential Oil. Packag. Food. Mach. 2018, 36, 25−30. DOI: 10.3969/j.issn.1005-1295.2018.06.005.

[18] Guo, G.; Yue, L.; Fan, S. L.; Jing, S. Q.; Yan, L. J. Antioxidant and Antiproliferative Activities of Portulaca Oleracea L. Seed Oil. Food. Sci. 2017, 38, 206−213. DOI: 10.7506/spkx1002-6630-201703034.

[19] Zeng, L.; Lin, Q. M.; Han, C. Y.; Zou, Y. P. Chemical Constituents, Antioxidant and Antibacterial Activities of Essential Oils from Torreya Grandis Leaf. J. Chin. Cereal. Oil. Ass. 2020, 25, 98–104.

[20] Yang, Z. J.; Zhou, Y.; Chen, L.; Zhao, Z.; Feng, T. T. A Preliminary Study on Chemical Constituents and Antioxidant Activity of the Volatile Oil from the Root of Cirsium Leducei (Franch.) Levl. J. Moun. Agri. Biol. 2014, 33, 32−34.

[21] Yuan, Y. F.; Hu, X. Y.; He, Y.; Deng, J. G. Synthesis and Anti-tumor Activity Evaluation of Rhein-Aloe Emodin Hybrid Molecule. Nat. Prod. Commun. 2012, 7, 207−210. DOI: 10.4155/FMC.11.184.

[22] Diego, B. V.; Gerardo, A. R.; Sandra, J. M.; Andrea, P.; Elena, I.; Fabián, P. A.; Alejandro, C. An Integrated Approach for the Valorization of Mango Seed Kernel: Efficient Extraction Solvent Selection, Phytochemical Profiling and Antiproliferative Activity Assessment. Food. Res. Int. 2019, 126, 1−14. DOI: 10.1016/j.foodres.2019.108016.

[23] Wang, C.; Li, R.; Wu, Y.; Wang, Y.; Tang, S. H.; Tan, J.; Jiang, Z. T. Rapid Screening of Radical Scavengers in Essential Oil from Piper Longum L. As Food Seasoning. Food. Sci. 2021, 14, 226−231. DOI: 10.7506/spkx1002-6630-20200701-016.

[24] Ayman, E. Anethole Inhibits the Proliferation of Human Prostate Cancer Cells via Induction of Cell Cycle Arrest and Apoptosis. Anticancer. Agents. Med. Chem. 2018, 2, 216−236. DOI: 10.2174/1871520617666170725165717.

[25] Flescher, E. Jasmonates in Cancer Therapy. Cancer. Lett. 2007, 245, 1−10. DOI: 10.1016/j.canlet.2006.03.001.

[26] Wang, K.; Liu, X.; Xu, H. R.; Qiu, F. Research Progress in Pharmacology, Pharmacokinetics and Toxicity of Natural Furancoumarins. Chin. J Pharmacol. Toxicol. 2021, 4, 312−320. DOI: 10.3867/j.1000-3002.2021.04.010.

[27] Zhang, N.; Chen, W. J.; Liu, Q. Q.; Zhou, Y.; Zhong, R. G. Studies on Radical Scavenging and Anti-tumor Effects of Coumarins. Food. Ré-D. 2016, 1, 1−5. DOI: 10.3969/j.issn.1005-6521.2016.01.001.

[28] Islam, S.; Samima Nasrin, S.; Khan, M. A.; Hussain, A. S.; Islam, F.; Khandokhar, P.; Mollah, M. N. H.; Rashid, M.; Sadik, G.; Rahman, M. A. A., et al. Evaluation of Antioxidant and Anticancer Properties of the Seed Extracts of Syzygium Fruticosum Roxb. Growing in Rajshahi, Bangladesh. BMC. Complement. Altern. Med. 2013, 13, 142−151. www.biomedcentral.com/1472-6882/13/142.

[29] Cheng, Y. C.; Xing, Y. Y.; Yang, S.; Shi, B. L. Research Progress on Biological Activity of Plant Volatile Oil and Application in Animal Production. Feed. Res. 2020, 12, 140−142. DOI: 10.13557/j.cnki.1002-2813.2020.12.037.