Materials Science for Energy Technologies 2 (2019) 349–355

Contents lists available at ScienceDirect

Materials Science for Energy Technologies

journal homepage: www.keaipublishing.com/en/journals/materials-science-

Extraction, characterization and antioxidant activity of fenugreek (*Trigonella-Foenum Graecum*) seed oil

Sweeta Akbari, Nour Hamid Abdurahman *, Rosli Mohd Yunus, Oluwaseun Ruth Alara, Olalere Olusegun Abayomi

Faculty of Chemical and Natural Resources Engineering, Universiti Malaysia Pahang, Lebuhraya Tun Razak, 26300, Gambang, Pahang, Malaysia

ARTICLE INFO

Article history:
Received 29 September 2018
Revised 3 December 2018
Accepted 21 December 2018
Available online 28 December 2018

Keywords:
Fenugreek seed oil
Gas chromatography-mass spectrometry
Unsaturated essential fatty acid
Antioxidant
Total phenolic content
DPPH
ABTS

ABSTRACT

Fenugreek (*Trigonella-Foenum Graecum*) is known as one of the traditional and most promising medicinal herbs belongs to the leguminous family. The seeds of fenugreek have been extensively studied for the treatment of inflammation, cancer and diabetes. In this study, fenugreek seed oil was extracted and evaluated for its chemical compositions and bonding through gas chromatography coupled to mass spectrometry (GC-MS) and Fourier transform infrared spectroscopy (FT-IR) analysis, respectively. The antioxidant activity against 2,2-diphenyl-picrylhydrazyl (DPPH) and 2,2′-Azino-bis-3-ethylbenzothiazoline-6-sulfonic acid ($ABTS^{+\bullet}$) radicals, total phenolic content (TPC) and total flavonoid content (TFC) of the oil were also studied. The capacity of antioxidants detected by ABTS was stronger than that by DPPH. A total number of 23 chemical compounds were detected and identified in fenugreek seed oil comprising of 99% of the total oil through GC-MS analysis. The major compounds of the extracted oil were linoleic acid (54.13%), palmitic acid (16.21%), pinene (4.56%), 4-Pentyl-1-(4-propylcyclohexyl)-1-cyclohexene (3.87%) and linoleic acid methyl ester (3.19%). FTIR analysis confirmed the presence of carboxyl group in the oil which were more dominated by unsaturated essential fatty acids. Moreover, the oil of fenugreek seed indicated a strong antioxidant radical scavenging activity against both DPPH and ABTS assays with an IC_{50} of 172.6 ± 3.1 and 161.3 ± 2.21, respectively. The TPC and TFC of the oil were 38.97 ± 0.34 mg GAE/g. oil and 14.417 ± 0.23 mg QE/g.oil. Thus, this study suggests that the fenugreek seed oil could be used for pharmaceutical purposes.

1. Introduction

Fenugreek (Trigonella-Foenum Graecum) is known as one of the traditional and most promising medicinal herbs belongs to the leguminous family. Historically, this plant has been widely used for more than 2500 years due to its food and medicinal properties as an herbal remedy [1]. Basically, it is reported to be native to West Africa and now extensively cultivated in Asia and Latin America as well [1,2]. The seeds and leaves of this plant are extensively employed in medicinal purposes as an anti-diabetic [3–5], anti-microbial [6] anti-inflammation [7], anti-cancer [8] and antioxidant agent [9–11]. Moreover, the seeds of fenugreek have also been reported to have strong free radical scavenging activity [12,13].

Free radicals are naturally produced in human body as a result of normal metabolism, different endogenous processes or exposure to some environmental pollutants. These radicals play an essential role in the biological functions of the entire body. However, due to their high reactivity as an oxidant and inhibitor of enzymes, they lead to the biomolecules oxidation such as protein, lipids, DNA and amino acids which finally result into cell damage and consequently cell death [14,15]. Thus, for a proper physiological function in the body, free radicals and antioxidants need to be balanced to avert oxidative stress. Oxidative stress is defined as the lack of balance between oxidants and antioxidants which could be linked to wide variety of diseases in human such as cancer, inflammation and diabetes [7]. Previous studies had reported that the dietary antioxidants are useful for protecting human body and preventing chronic illnesses [14]. Generally, there are two types of antioxidants used in health-related industries: natural and synthetics.

* Corresponding author.
E-mail address: abrahman@ump.edu.my (N.H. Abdurahman).

Peer review under responsibility of KeAi Communications Co., Ltd.

Production and hosting by Elsevier

https://doi.org/10.1016/j.mset.2018.12.001

The very known synthetic antioxidants including butylated hydroxyanisole (BHA) and butylhydroxytoluene (BHT) are potentially suspected to have harmful effects to human health, they can result in liver damage and also possess carcinogenic effects [16]. Recently, interests have been increased in natural antioxidants produced from many species of fruits, seeds, cereals, vegetables and plants [17–20]. Natural antioxidants are well known for their protective capacity in living organisms and cells against free radical attacks [21]. Natural antioxidants could be advantageous over synthetic antioxidants as they do not contain chemicals and also been components of human diet for thousand years.

The use of essential fatty acids and oils as promising and natural antioxidants are achieving a growing interest in food, cosmetic and pharmaceutical products because of their potential health benefits and harmless effects. The evaluation of their antioxidant performance made them a valid alternative to synthetic antioxidants such as BHA and BHT [16]. Essential fatty acids are unsaturated lipids that cannot be synthesized in the body and must be acquired from external sources. The two essential polyunsaturated fatty acids (PUFAs) namely linoleic acid (omega-6 fatty acids) and alpha-linolenic acid (omega-3 fatty acids) are considered very important for human body to survive. Studies have shown that essential fatty acids can treat certain diseases such as cancers and diabetes [22]. Particularly, linoleic acid is recognized as the essential nutrient for humans. Linoleic acid is mostly found in walnut oil, sunflower oil, cottonseed oil, pumpkin seed oil, coconut oil and many leafy vegetables [22,23]. In fact, essential fatty acids and oils can be produced from several parts of the plants such as leaves, fruits, roots, seeds, wood, stems, and shells using different solvents (n-hexane, chloroform, diethyl ether, acetone and ethanol). Commonly, n-hexane has been extensively used to extract the essential oils of plants [24,25]. In essence, plant extracts and oils have not only used for the purpose of antioxidant stability in foods, drinks, cosmetics creams and other goods, but they are also used as multi-purpose additive such as flavouring, anti-microbial and anti-fungal agents [26–28]. To date, one of the plants that is well known for its medicinal values and have been used since the ancient time as a food source or herbal remedy is fenugreek. Different studies have reported the potential of antioxidant capacity and other phytochemical activity in fenugreek seed extracts [10,13]. However, a little is known about the capacity of antioxidant activity and total phenolic content of its extracted oil. Therefore, this study is undertaken to determine the chemical properties of the oil extracted from fenugreek seed and to evaluate its antioxidant activity based on DPPH and FRAP assays, total phenolic content and total flavonoid content as well.

2. Materials and methods

2.1. Procurement of fenugreek seed

Seeds of fenugreek were purchased from local retail market in Kuantan, Pahang, Malaysia. The seeds were cleaned before drying it in oven at 50 °C for 24 h. Then, the dried seeds were ground using a mill with ultra-centrifugal (Retsch ZM-200, Germany) equipped with ring sieve owning trapezoid holes sized 0.5 mm. The moisture content of the seed was (5.51 ± 0.14% d.w basis). The powdered seeds were kept in dark airtight container before extraction.

2.2. Analytical reagents and chemicals

Methanol (99% purity), n-hexane (99% purity), 2,2-diphenylpicrylhydrazyl (DPPH), sodium carbonate, Folin-Ciocateu reagent and gallic acid (GA) were obtained from Sigma Aldrich (M) Sdn. Bhd, Selangore, Malaysia. All chemicals used for the extraction process were analytical grade with high purity.

2.3. Extraction process

A 100 g of crushed fenugreek seed was extracted using n-hexane (600 mL) and a Soxhlet extractor for 3 h at (65–70 °C). Then, the mixture of solvent-oil was filtered through a No.1 paper filter (Whatman). The extract was transferred into a round flask and solvent was evaporated using rotary evaporator, (Rotavapor R-200, Büchi, Germany) at 40 °C. Finally, the oil extract was stored at 4 °C to prevent degradation of the compounds for further analysis. The yield of extraction was calculated using Eq. (1).

$$\text{Extraction yield } (\frac{v}{w}\%) = \frac{\text{Amount of oil extracted } (mL)}{\text{Weight of dry sample used } (g)} x\ 100 \quad (1)$$

2.4. GC-MS analysis of the oil

The GC-MS analysis of extracted oil was accomplished using a GC-MS (Agilent 5973- model, USA) with C-18 column (30 mm diameter of tubular column, 0.25-mm internal diameter and 0.25-μm thickness of film). Initially, the oven temperature was kept stable at 60 °C for 4 min and then allowed to rise at a rate of 6 °C/min to 230 °C. The temperatures of injector and detector were set at 230 °C and 260 °C, respectively. Helium gas was applied as carrier at a flow rate of 1 mL/min. The oil sample was filtered using microfilter 0.45 μm and mixed with n-hexane at ratio of 1:10 then injected by split injection. The identification of the chemical compounds of the oil was determined by matching the obtained mass spectra of the sample with the mass spectra report provided by the library of National Institute of Standards and Technology (NIST05.LIB).

2.5. Fourier transform infrared spectroscopy (FT-IR)

The determination of functional groups and bonding structure of fenugreek seeds oil were determined using Thermo Scientific Nicolet iS5 spectrometer equipped with deuterated triglycine sulfate (DTGS) as a detector and using Potassium bromide (KBr) methods. IR spectra were measured in the range of 400 to 4000 cm^{-1} at 4 cm^{-1} selected resolution and room temperature 25 °C. Approximately, one drop of oil was placed on the surface of the diamond crystal to form a thin film for analysis of infrared spectrometry and measured directly.

2.6. DPPH free radical scavenging activity

Antioxidant activity of fenugreek seed oil was measured using the method explained by Alara *et al.* [29] with slight modifications. Concisely, 0.2 mL of various concentrations of extract in methanol were separately added to 2 mL of DPPH (0.1 mM) solution. The mixture was vigorously shaken and then incubated for 30 min in a dark drawer at room temperature. Then the absorbance of the sample was measured at a wavelength of 517 nm using a UV–Vis spectrophotometer (Hitachi U-1800, Japan). Methanol was applied as the blank. The scavenging activity on DPPH was calculated using Eq. (2). The half maximal inhibitory concentration (IC_{50}) of the sample was also calculated to find the inhibition percentage against oil concentration.

$$\text{DPPH radical scavenging activity } (\%) = \frac{A_{control} - A_{sample}}{A_{control}} x\ 100 \quad (2)$$

where $A_{control}$ shows the absorbance of DPPH and methanol solution without sample, and A_{sample} represents the mixture of sample (extracted oil) and solution of DPPH.

2.7. ABTS free radical scavenging activity

The radical scavenging capacity of fenugreek oil was also examined using ABTS assay. This assay, followed the procedure described by Zielinski *et al.* [30] with some modifications. Initially, the stock solutions of 7 mM ABTS and 2.45 mM potassium persulfate ($K_2S_2O_8$) were separately prepared by dissolving them in distilled water. Then, the working solution was prepared by mixing the equal quantities of the two stock solutions and allowed to rest for 12–16 h in a dark place at room temperature. Further, a fresh working solution was prepared by mixing 1 mL of ABTS solution diluting in 60 mL of methanol to obtain a constant absorbance of 1.1 ± 0.02 at wavelength of 734 nm using a UV–vis Spectrophotometer (Hitachi U-1800, Japan). Thereafter, 150 μL of the fenugreek seed oil was mixed with 2.85 mL of ABTS solution and the absorbance was taken at 734 nm after incubating in a dark place for 2 h. Methanol was used as the blank and the free radical scavenging activity of the oil was calculated using Eq. (3).

$$ABTS\,radical\,scavenging\,activity\ (\%) = \frac{A_{control} - A_{sample}}{A_{control}} x\ 100 \qquad (3)$$

where $A_{control}$ shows the absorbance of ABTS and methanol solution without sample, and A_{sample} represents the mixture of oil and ATBS solution.

2.8. Total phenolic content (TPC) of the oil

The determination of TPC of the extracted oil was carried out based on the methods explained by Sarikurkcu *et al.* [26] and Iness *et al.* [31] with slight modifications. Concisely, 1 mL of the oil was mixed with 0.2 mL of Folin-Ciocalteu reagent and 2 mL of methanol was shaken thoroughly, before allowing to rest for 5 min in the dark place at room temperature. Then, 1 mL of 20% (w/v) solution prepared from sodium carbonate (Na_2CO_3) was added and incubated in a drawer for the next 2 h. Thereafter, the absorbance of the sample was measured at 765 nm using UV–Vis spectrophotometry (Hitachi U-1800, Japan). The concentration of sample was calculated and obtained from the standard curve of gallic acid (100–500 mg/mL) equation ($Y = 0.0003x + 0.0391$; $R^2 = 0.9662$) equation and the results were expressed as mg gallic acid equivalent per gram of oil (mg GAE/g. oil). The total phenolic content of the extract was obtained based on Eq. (4). Methanol was used as blank versus the prepared sample.

$$TPC = \frac{c \times V}{m} \qquad (4)$$

where in the above equation c represents the concentration of the sample taken from the gallic acid calibration curve (mg/mL), V is the solvent (n-hexane) volume used for the extraction of oil (mL), and m indicates the weight (g) of the extracted oil.

2.9. Total flavonoid content (TFC) of the oil

The determination of total flavonoid content in fenugreek seed oil was performed based on the methods described by Dahmoune *et al.* [32] and Alara *et al.* [18] with some modifications. concisely, 1 mL of extracted oil was added into a glass test tube. Then, 1 mL of 2% ethanolic $AlCl_3$ was added and incubated in the dark for 30 min at room temperature. The absorbance was measured at 415 nm using a UV–vis Spectrophotometer (Hitachi U-1800, Japan). The concentration of total flavonoid content of the oil was then measured using quercetin standard curve (100–600 mg/mL) plotted with the equation of ($y = 0.0006x + 0.1523$; $R^2 = 0.9923$). Where y shows the absorbance at 415 nm and x indicates the sample concentration in mg/mL. The results were expressed in mg quercetin equivalent (QE) per g of dry sample (mg QE/g d.w). The total flavonoid content of the oil was determined based on Eq. (5). Methanol was used as blank versus the prepared sample.

$$TFC = \frac{c \times V}{m} \qquad (5)$$

where in this equation c indicates the concentration of the sample taken from the quercetin calibration curve (mg/mL), V is the solvent volume used for the extraction of oil (mL), and m indicates the weight (g) of the extracted oil.

3. Results and discussion

3.1. Extraction and GC-MS characterization of fenugreek seed oil

The oil of fenugreek seed was extracted using Soxhlet extraction technique and n-hexane as the extraction solvent. The seeds were yielded 5.55 ± 0.05% (v/w) oil having a yellowish colour. The extracted oil was characterized through the GC-MS analysis. A total number and 23 compounds were identified with a total of 99% compositions. Table 1 represents the detected constituents along with their molecular formula, retention time (RT), percentage of composition and pharmacological activities obtained from PubChem. Fig. 1 displays the GC-MS characterization of fenugreek seed oil with respect to retention time (min) and abundance of compounds. These results indicated that the major compounds in fenugreek seed oil were linoleic acid (54.13%), palmitic acid (16.21%), pinene (4.56%), 4-Pentyl-1-(4-propylcyclohexyl)-1-cyclohexene (3.87%), and linoleic acid methyl ester (3.19%). The chemical composition of the extracted oil obtained in this study was slightly different from the previous studies, which can be attributed to the type of extraction method and the type of solvents used to extract the oil. However, some compounds detected in this study were similar to the previous findings. For example, Gu et al. [33] obtained linoleic acid as the dominated compound in the oil, contributing approximately 42% of the total oil composition, similar to this study that also obtained linoleic acid (54.13%) as the major compound in the oil. Similarly, Sulieman *et al.* [34] extracted the oil of fenugreek seed by homogenizing the seed powder with 2:1 chloroform-methanol (v/v), which also reported linoleic acid (43.2%) as the dominated compound in the oil. Overall, the derived oil of fenugreek seed consisted of the compounds such as esters, alkanes, saturated and unsaturated fatty acids, glycerides and other related compounds such as phenols, flavonoids and alkaloids.

Fig. 2 shows the extracted fenugreek seed oil possessed two main constituents found in the oil, linoleic acid and palmitic acid. Linoleic acid is known as polyunsaturated omega-6 fatty acid, having 18-carbon chain with twin bonds in cis configuration. Studies revealed that linoleic acid has been recognized as an important diet in human body due to its strong anticancer activity especially in terms of breast cancer treatment [35,36]. The second highest compound (palmitic acid) is known for its biological and antioxidant activity. Palmitic acid is known as one of the most common saturated fatty acid with 16 carbon backbone found in plants, animals, and microorganisms. It has been also used as a food additive and anti-inflammation, its daily intake may lead to a healthier life [37]. The other compounds with similar biological activity such as pinene, linoleic acid methyl ester, pentadance and phytol have a wide range of pharmacological activities as an antioxidant, anti-ashtmatic, and also used as drug for sexual and urinary disorders [38–41]. The presence of these compounds along with palmitic acid could be a possible reason for antioxidant activity of fenugreek seed oil [42]. In addition, most of the identified compounds of the extracted oil exhibited antiasthmatic, and considerable effects against sexual and urinary disorders. Therefore, it could be suggested that the oil extracted in this study could be used in pharmaceutical industries for various medical purposes.

Table 1
GCMS chemical composition of fenugreek seed oil with their pharmacological activities referenced from PubChem.

Peak #	Constituents	Molecular formula	R. time	Composition (%)	Pharmacological activity/Application	PubChem CID	References
1	2-Methylpyrrolidine	$C_5H_{11}N$	9.768	0.42	Have a wide range of biological activity	13,003	[43]
2	Dodecane	$C_{12}H_{26}$	14.004	0.22	Cosmetic solvent, bioactive, also used to assess lipid peroxidation in asthmatic patients	8182	[44,45]
3	α-terpinene	$C_{10}H_{16}$	14.854	0.32	Antioxidant, antiasthmatics, antibacterial agents, for wound treatment, food, flavouring agent, also used for urinary and sexual disorder, bioactive	7462	[46,47]
4	α -Terpinyl acetate	$C_{12}H_{20}O_2$	17.63	0.41	Antioxidant, antibiotic, antiseptic, drug for disorders of the nervous system, also used as food additive, bioactive	111,037	[48,49]
5	Tetradecane	$C_{14}H_{30}$	18.63	0.33	Antioxidant, antiasthmatics, drugs for sexual and urinary disorders, also used for throat disorders, bioactive	12,389	[40,50]
6	Caryophyllene	$C_{15}H_{24}$	19.63	0.32	Essential oil, used in food products, bioactive	5,281,515	[51]
7	Pentadecane	$C_{15}H_{32}$	21.973	2.06	Antioxidant, antiasthmatics, antibacterial, used in food products, also used for urinary system and sexual disorder in both male and female	12,391	[40,50,52]
8	Trichloroacetic acid, pentadecyl ester	$C_{17}H_{31}C_{l3}O_2$	27.798	1.45	Used in cosmetic treatments	522,535	[53]
9	Pinene	$C_{10}H_{18}$	29.317	4.56	Antioxidant, antiasthmatics, antibacterial, for sexual and urinary system disorders, and also used for enhancing fertility, bioactive	10,129	[38,54]
10	Phytol	$C_{20}H_{40}O$	30.14	1.78	Antioxidant, antiasthmatics, anti-inflammatory, anti-cancer, antibacterial, used in body care products and making synthetic forms of vitamin E and vitamin K1, also used for sexual and urinary system disorder, bioactive	5,280,435	[41,55,56]
11	Nonadecane	$C_{19}H_{40}$	30.6	1.31	No activity was recorded	12,401	
12	Palmitic acid or n-Hexadecanoic acid	$C_{16}H_{32}O_2$	31.847	16.21	Antioxidant, anticancer, food additive, anti-inflammation	985	[57,58]
13	Eicosane	$C_{20}H_{42}$	32.505	1.61	Antioxidant, anticancer, antitumor, antibacterial agents	8222	[52,59]
14	Linoleic acid methyl ester	$C_{19}H_{34}O_2$	33.804	3.19	Antioxidant, antibacterial agent, also useful for sexual and urinary system disorder	5,284,421	[39]
15	Linoleic acid	$C_{18}H_{32}O_2$	35.04	54.13	Antioxidant, used for cancer treatment, anti-diabetes, antibacterial agent, essential fatty acid, also used for increasing sex hormone in men	3931	[23,60,61]
16	4-Pentyl-1-(4-propylcyclohexyl)-1-cyclohexene	$C_{20}H_{36}$	36.174	3.87	No activity was recorded	557,007	
17	1-Piperidinepropanenitrile	$C_8H_{14}N_2$	37.436	2.76	Antibiotics, also used for DNA studies	18,338	[62,63]
18	Palmidrol	$C_{18}H_{37}NO_2$	38.158	0.68	Free radical scavengers or antioxidants, antiallergic agent, anti-inflammation, skin treatment, anticancer, drugs for disorders of the urinary system, also used for genital or sexual disorders, bioactive	4671	[64,65]
19	Arachidic Acid or Eicosanoic acid	$C_{20}H_{40}O_2$	38.8	0.84	Saturated fatty acid, antiasthmatics, antibacterial agents, antioxidants, anticancer, also used for urinary or sexual disorders treatment.	10,467	[66–68]
20	Glyceryl 2-linoleate	$C_{21}H_{38}O_4$	39.506	1.11	Antioxidant, antibiotics, anti-inflammatory agent, also used as emulsifier.	5,365,676	[69]
21	(R)-(-)-(Z)-14-Methyl-8-hexadecen-1-ol	$C_{17}H_{34}O$	40.667	0.64	Plant growth regulator, also used as Pheromones	12,487,634	[70]
22	Quinoline	C_9H_7N	41.71	0.75	Antitussive agent, antiasthmatic and Antibacterial agent, also used for urinary disorder, bioactive.	7047	[71,72]
23	Tetratriacontane	$C_{34}H_{70}$	42.581	0.03	Antioxidant, antimicrobial agent, bioactive	26,519	[73]

3.2. Fourier transform infrared spectroscopy of the oil

The functional groups of fenugreek seed oil were obtained by FTIR spectra as shown in Fig. 3. The strong absorption peaks at 2853.16–3009.19 cm^{-1} are assigned to both symmetric and asymmetric stretching of methyl (-CH_3) and methylene (-CH_2) groups which can be attributed to the existence of fatty acids and their methyl esters within the carboxyl groups (COOH). The peaks at 1743.61 cm^{-1} corresponds to the saturated fat aldehyde. The unique band at 1460.30 cm^{-1} represents the alcoholic (C-OH) group. The aromatic acid esters (C-O-C) and the stretching vibration of phenolic compounds (C-OH) were observed at 1098.14 to 1236.89 cm^{-1}, they can also be referred to amide groups. Finally, the peak at 721.15 cm^{-1} can be assigned to a benzene ring [74]. Based on the FTIR spectral result obtained, different functional groups were existed in seed oil of fenugreek such as carboxyl, hydroxyl, fats, alcohols, amides and phenolic compounds.

3.3. DPPH radical scavenging activity of the oil

The potential of antioxidant activity in fenugreek seed oil was determined using DPPH scavenging essay. The DPPH radical-scavenging test is a commonly employed assay in antioxidant studies. The antioxidant effects of plant extracts on DPPH radical scavenging may be due to their hydrogen-donating abilities, which reduce the stable violet DPPH radical to the yellow DPPH-H. The potential of free radical scavenging can be indicated by the degree of discolouration of the sample or extract. In the present study, the degree of antioxidant activity of oil is given based on the 50% inhibition concentration (IC_{50}). The fenugreek seed oil indicated an IC_{50} equal to 172.6 ± 3.1 μg/mL (Table 2). This value shows a good antioxidant activity due to the existence of palmitic acid and phytol detected by GC-MS analysis as they are well known for their antioxidant and some other biological activities. Moreover, the oil of fenugreek seed has also shown some compounds with anti-inflammation activities [37,75].

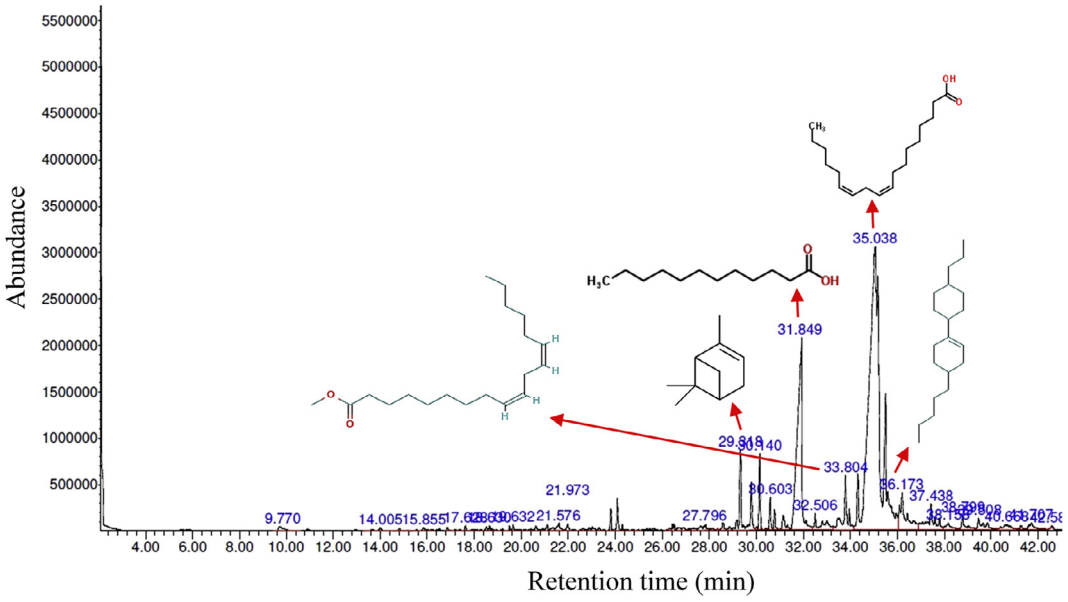

Fig. 1. GC-MS characterization of fenugreek seed oil.

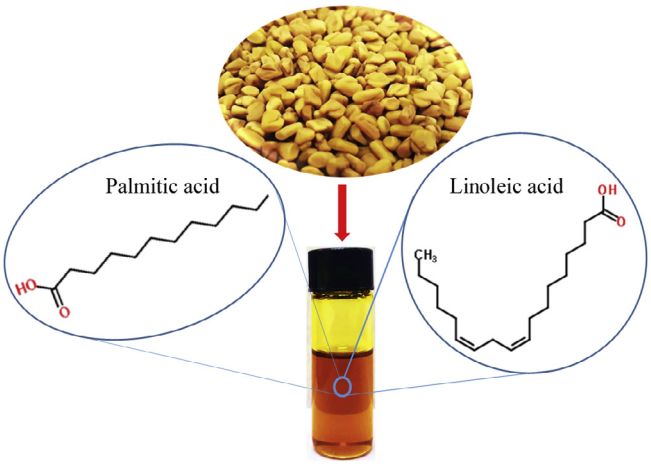

Fig. 2. Fenugreek seed oil with the structures of two major compounds identified in the oil.

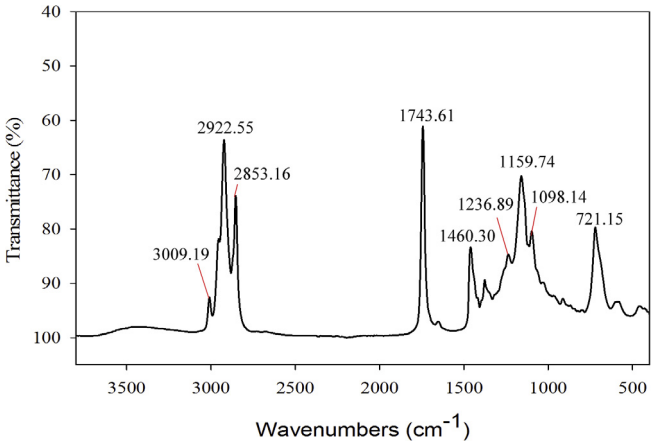

Fig. 3. FTIR spectrum of fenugreek seed oil.

3.4. ABTS radical scavenging activity of the oil

The antioxidant activity of the oil was also examined using ABTS assay. As it can be seen in Table 2 that antioxidant capacity of the oil by ABTS assay (IC_{50} = 161.3 ± 2.21 μg/mL) was stronger than that by DPPH assay (IC_{50} = 172.6 ± 3.1 μg/mL). Lower IC_{50} value means higher antioxidant activity. Both DPPH and ABTS compounds have proton free radicals that significantly decreased when exposed to proton radical scavengers [76]. It can be suggested that most of plant compounds show better antioxidant activity against ABTS radicals than DPPH radicals. This is due to more sensitivity of ABTS assay in identifying antioxidant activity which makes the kinetic reaction faster and hence results into the higher antioxidant activity [76,77].

3.5. Total phenolic content of fenugreek seed oil

The determination of TPC in plant seeds oil are considered necessary as one of the important indicators of oil quality is phenolic compound. These compounds are responsible for the capacity of free radical scavenging and peroxidation of lipids. In this study, TPC was determined using Folin- Ciocalteau assay and the result expressed as gallic acid equivalent. The fenugreek seed oil showed phenolic content of 38.97 ± 0.34 mg/g equivalent to gallic acid (Table 2). Whereas, this result is in a good agreement with that found by Arora and Pandey-rai [75]. The results indicated a positive relevance between antioxidant activity of the oil and its total phenolic contents.

3.6. Total flavonoids content of fenugreek seed oil

Beside the major contribution of phenolic compounds in antioxidant capacity of the foods, flavonoids have also shown to have free radical scavenging activity that can be considered significant in human diet. In this study, the total flavonoid content of fenugreek seed oil was measured using spectrophotometric method. Quercetin was used as a standard for total flavonoid content measurement. Fenugreek seed oil indicated a total flavonoid content of 14.417 ± 0.23 mg QE/g.oil as shown in Table 2. These results support the good antioxidant capacity of fenugreek seed oil. High content of phenolic and flavonoid compounds means higher antioxidant activity of the plants [78].

Table 2
Summary of extraction yield, DPPH, ABTS, TPC and TFC of fenugreek seed oil.

Sample	Extracted Yield/oil (%)	IC_{50} of DPPH (μg/mL)	IC_{50} of ABTS (μg/mL)	TPC (mg GAE/g.oil)	TFC (mg QE/g.oil)
Fenugreek seed	5.55 ± 0.05	172.6 ± 3.1	161.3 ± 2.21	38.97 ± 0.34	14.417 ± 0.23

Values are expressed as the mean standard deviation (±SD) where (n = 3).

4. Conclusion

In this study, fenugreek seed oil was extracted using Soxhlet extraction technique and n-hexane as extraction solvent. The GC-MS and FTIR analysis of fenugreek seed oil revealed that the oil is rich in essential omega-6 fatty acids (linoleic acid), which are highly effective for prevention of coronary heart diseases, inflammation and cancer. In addition, the main constituents of the oil namely, linoleic acid, palmitic acid, pinene and other components with lower portions were found to be very useful in reducing free radicals due to their natural antioxidant properties. This study also demonstrated that ABTS radical scavenging assay was more useful as compared to DPPH assay. Overall, based on the results obtained in this study it could be suggested that fenugreek seed oil could be effective against many diseases such as cancer, inflammation, asthma, sexual disorder and urinary infections.

Conflict of interest

The authors declare no financial interest in this manuscript.

Acknowledgements

All authors are grateful to Faculty of Chemical and Natural Resources Engineering for the laboratory and financial supports provided by Universiti Malaysia Pahang (UMP) internal grant (PGRS 1803105) in this project.

References

[1] X. Shi, T. Miyakawa, A. Nakamura, F. Hou, M. Hibi, J. Ogawa, Y. Kwon, M. Tanokura, Engineering a short-chain dehydrogenase/reductase for the stereoselective production of (2S,3R,4S)-4-hydroxyisoleucine with three asymmetric centers, Sci. Rep. 7 (2017) 1–13, https://doi.org/10.1038/s41598-017-13978-w.
[2] J.X. Jiang, L.W. Zhu, W.M. Zhang, R.C. Sun, Characterization of galactomannan gum from fenugreek (trigonella foenum-graecum) seeds and its rheological properties, Int. J. Polym. Mater. Polym. Biomater. 56 (2007) 1145–1154, https://doi.org/10.1080/00914030701323745.
[3] M. Bahmani, H. Shirzad, M. Mirhosseini, A. Mesripour, M. Rafieian-Kopaei, A Review on ethnobotanical and therapeutic uses of fenugreek (Trigonella foenum-graceum L), J. Evidence-Based Complement. Altern. Med. 21 (2016) 53–62, https://doi.org/10.1177/2156587215583405.
[4] A. Swaroop, M. Bagchi, H.G. Preuss, D. Bagchi, Safety and antidiabetic efficacy of a novel Trigonella foenum-graecum seed extract, 2nd Ed, Elsevier Inc., 2018. doi:10.1016/B978-0-12-812019-4.00028-3.
[5] B. Annida, P.S.M. Prince, Supplementation of fenugreek leaves reduces oxidative stress in streptozotocin-induced diabetic rats, J. Med. Food. 8 (2005) 382–385.
[6] S. Subhapriya, P. Gomathipriya, Green synthesis of titanium dioxide (TiO_2) nanoparticles by Trigonella foenum-graecum extract and its antimicrobial properties, Microb. Pathog. 116 (2018) 215–220, https://doi.org/10.1016/j.micpath.2018.01.027.
[7] R. Tavakoly, M.R. Maracy, M. Karimifar, M.H. Entezari, Does fenugreek (Trigonella foenum-graecum) seed improve inflammation, and oxidative stress in patients with type 2 diabetes mellitus? A parallel group randomized clinical trial, Eur. J. Integr. Med. 18 (2018) 13–17, https://doi.org/10.1016/j.eujim.2018.01.005.
[8] K. El Bairi, M. Ouzir, N. Agnieszka, L. Khalki, Anticancer potential of Trigonella foenum graecum: cellular and molecular targets, Biomed. Pharmacother. 90 (2017) 479–491, https://doi.org/10.1016/j.biopha.2017.03.071.
[9] T. Mehmood, M. Ahmed, Z. Jabeen, A. Karim, M.A. Shaheen, F. Siddique, Attributes of bioactive compounds isolated from commercial brands of fenugreek (Trigonella foneum-graecum) in relation to organic solvent systems and their potential as antioxidants and biological activity, Pure Appl. Biol. 6 (2017) 871–881.
[10] P. Mukthamba, K. Srinivasan, Dietary fenugreek (Trigonella foenum-graecum) seeds and garlic (Allium sativum) alleviates oxidative stress in experimental myocardial infarction, Food Sci. Hum. Wellness. 6 (2017) 77–87, https://doi.org/10.1016/j.fshw.2017.04.001.
[11] S.A. Wani, S. Bishnoi, P. Kumar, Ultrasound and microwave assisted extraction of diosgenin from fenugreek seed and fenugreek-supplemented cookies, J. Food Meas. Charact. 10 (2016) 527–532, https://doi.org/10.1007/s11694-016-9331-2.
[12] S. Chatterjee, P.S. Variyar, A. Sharma, Stability of lipid constituents in radiation processed fenugreek seeds and turmeric: role of phenolic antioxidants, J. Agric. Food Chem. 57 (2009) 9226–9233, https://doi.org/10.1021/jf901642e.
[13] W.N. Baba, Q. Tabasum, S. Muzzaffar, F.A. Masoodi, I. Wani, S.A. Ganie, M.M. Bhat, Some nutraceutical properties of fenugreek seeds and shoots (Trigonella foenum-graecum L.) from the high Himalayan region, Food Biosci. 23 (2018) 31–37, https://doi.org/10.1016/j.fbio.2018.02.009.
[14] Y.-Z. Fang, S. Yang, G. Wu, Free radicals, antioxidants, and nutrition, Nutrition 18 (2002) 872–879, https://doi.org/10.1016/S0899-9007(02)00916-4.
[15] V. Lobo, A. Patil, A. Phatak, N. Chandra, Free radicals, antioxidants and functional foods: Impact on human health, Pharmacogn. Mag. 4 (2010) 118–126, https://doi.org/10.4103/0973-7847.70902.
[16] R. Amorati, M.C. Foti, L. Valgimigli, Antioxidant activity of essential oils, J. Agric. Food Chem. 61 (2013) 10835–10847, https://doi.org/10.1021/jf403496k.
[17] S. Dudonné, X. Vitrac, P. Coutiére, M. Woillez, J.M. Mérillon, Comparative study of antioxidant properties and total phenolic content of 30 plant extracts of industrial interest using DPPH, ABTS, FRAP, SOD, and ORAC assays, J. Agric. Food Chem. 57 (2009) 1768–1774, https://doi.org/10.1021/jf803011r.
[18] O.R. Alara, N.H. Abdurahman, O.A. Olalere, Ethanolic extraction of flavonoids, phenolics and antioxidants from Vernonia amygdalina leaf using two-level factorial design, J. King Saud Univ. - Sci. (2017) 1–10, https://doi.org/10.1016/j.jksus.2017.08.001.
[19] S. Chaturvedi, R. Hemamalini, S.K. Khare, Effect of processing conditions on saponin content and antioxidant activity of Indian varieties of soybean (Glycine max Linn.), Ann. Phytomed. 1 (2012) 62–68.
[20] R.P.T. Kim, V. Bihud, K. Bin Mohamad, K.H. Leong, J. Bin Mohamad, F. Bin Ahmad, H. Hazni, N. Kasim, S.N.A. Halim, K. Awang, Cytotoxic and antioxidant compounds from the stem bark of Goniothalamus tapisoides mat salleh, Molecules 18 (2013) 128–139, https://doi.org/10.3390/molecules18010128.
[21] H.S.A. Seif, Physiological changes due to hepatotoxicity and the protective role of some medicinal plants, Beni-Suef Univ. J. Basic Appl. Sci. 5 (2016) 134–146, https://doi.org/10.1016/j.bjbas.2016.03.004.
[22] J. Orsavova, L. Misurcova, J.V. Ambrozova, R. Vicha, Fatty acids composition of vegetable oils and its contribution to dietary energy intake and dependence of cardiovascular mortality on dietary intake of fatty acids, Mol. Sci. 16 (2015) 12871–12890, https://doi.org/10.3390/ijms160612871.
[23] D. Liang, Y. Hu, W. Ma, Z. Zhao, S. Jiang, Y. Wang, X. Zhang, Concentration of linoleic acid from cottonseed oil by starch complexation, Chinese J. Chem. Eng. (2018), https://doi.org/10.1016/j.cjche.2018.07.001.
[24] M. Olszowy, A.L. Dawidowicz, Essential oils as antioxidants: their evaluation by DPPH, ABTS, FRAP, CUPRAC, and β-carotene bleaching methods, Monatshefte Fur Chemie. 147 (2016) 2083–2091, https://doi.org/10.1007/s00706-016-1837-0.
[25] S. Feyzi, M. Varidi, F. Zare, M.J. Varidi, A comparison of chemical, structural and functional properties of fenugreek (Trigonella foenum graecum) protein isolates produced using different defatting solvents, Int. J. Biol. Macromol. 105 (2017) 27–35, https://doi.org/10.1016/j.ijbiomac.2017.06.101.
[26] C. Sarikurkcu, B. Tepe, D. Daferera, M. Polissiou, Studies on the antioxidant activity of the essential oil and methanol extract of Marrubium globosum subsp. globosum (lamiaceae) by three different chemical assays, Bioresour. Technol. 99 (2008) 4239–4246, https://doi.org/10.1016/j.biortech.2007.08.058.
[27] G.S. El-Baroty, H.H.A. El-Baky, R. Farag, M. Saleh, Characterization of antioxidant and antimicrobial compounds of cinnamon and ginger essential oils, African J. Biochem. Res. 4 (2010) 167–174.
[28] M. Mehani, L. Segni, Antimicrobial effect of essential oil of plant trigonella focnum greacum on some bacteria pathogens, World Acad. Sci. Eng. Technol. 7 (2012) 358–360.
[29] O.R. Alara, N.H. Abdurahman, C.I. Ukaegbu, N.H. Azhari, Vernonia cinerea leaves as the source of phenolic compounds, antioxidants, and anti-diabetic activity using microwave-assisted extraction technique, Ind. Crops Prod. 122 (2018) 533–544, https://doi.org/10.1016/j.indcrop.2018.06.034.
[30] A.F. Zielinski, M.V. Silva, P.V.D.A. Pontes, S.R.F. Iora, G.M. Maciel, C.W.I. Haminiuk, D. Granato, Original article ewvaluation of the bioactive compounds and the antioxidant capacity of grape pomace, Int. J. Food Sci. Technol. 50 (2014) 62–69, https://doi.org/10.1111/ijfs.12583.
[31] B. Iness Bettaieb, Soumaya Bourgou, Wissem Aidiwannes, Ibtissem Hamrouni, Ferid Limam, Essential oils, phenolics, and antioxidant activities of different

parts of cumin (Cuminum cyminum L.), J. Agric. Food Chem. 58 (2010) 10410–10418, https://doi.org/10.1021/jf102248j.
[32] F. Dahmoune, B. Nayak, K. Moussi, H. Remini, K. Madani, Optimization of microwave-assisted extraction of polyphenols from Myrtus communis L. leaves, Food Chem. 166 (2015) 585–595, https://doi.org/10.1016/j.foodchem.2014.06.066.
[33] L.B. Gu, X.N. Liu, H.M. Liu, H.L. Pang, G.Y. Qin, Extraction of fenugreek (Trigonella foenum-graceum L.) seed oil using subcritical butane: characterization and process optimization, Molecules 22 (2017) 2–14. doi:10.3390/molecules22020228.
[34] A.M.E. Sulieman, A.O. Ali, J. Hemavathy, Short communication Lipid content and fatty acid composition of fenugreek (Trigonella foenum-graecum L.) seeds grown in Sudan, Int. J. Food Sci. Technol. 43 (2008) 380–382, https://doi.org/10.1111/j.1365-2621.2006.01446.x.
[35] X.M. Tao, J.C. Wang, J.B. Wang, Q. Feng, S.Y. Gao, L.R. Zhang, Q. Zhang, Enhanced anticancer activity of gemcitabine coupling with conjugated linoleic acid against human breast cancer in vitro and in vivo, Eur. J. Pharm. Biopharm. 82 (2012) 401–409, https://doi.org/10.1016/j.ejpb.2012.06.007.
[36] J. Iqbal, B.A. Abbasi, T. Mahmood, S. Kanwal, B. Ali, S.A. Shah, A.T. Khalil, Plant-derived anticancer agents: a green anticancer approach, Asian Pac. J. Trop. Biomed. 7 (2017) 1129–1150, https://doi.org/10.1016/j.apjtb.2017.10.016.
[37] B. Benzidia, M. Barbouchi, H. Hammouch, N. Belahbib, M. Zouarhi, H. Erramli, N. Ait Daoud, N. Badrane, N. Hajjaji, Chemical composition and antioxidant activity of tannins extract from green rind of Aloe vera (L.) Burm. F., J. King Saud Univ. - Sci. (2018), https://doi.org/10.1016/j.jksus.2018.05.022.
[38] A.D. Gupta, Chemistry, antioxidant and antimicrobial potential of nutmeg (Myristica fragrans Houtt), J. Genet. Eng. Biotechnol. 11 (2013) 25–31, https://doi.org/10.1016/j.jgeb.2012.12.001.
[39] G. Agoramoorthy, M. Chandrasekaran, V. Venkatesalu, M.J. Hsu, Antibacterial and antifungal activities of fatty acid methyl esters of the blind-your-eye mangrove from India, Brazilian J. Microbiol. 38 (2007) 739–742.
[40] A. Costanzo, S. Panseri, A. Giorgi, A. Romano, M. Caprioli, N. Saino, The odour of sex: Sex-related differences in volatile compound composition among barn swallow eggs carrying embryos of either sex, PLoS One. 11 (2016) 1–17, https://doi.org/10.1371/journal.pone.0165055.
[41] R.O. Silvaa, N.S.C. Francisca Beatriz M. Sousaa, Samara R.B. Damascenoa, Phytol, a diterpene alcohol, inhibits the inflammatory response by reducing cytokine production and oxidative stress Phytol inhibits the inflammatory response, Fundam. Clin. Pharmacol. 28 (2014) 455–464. doi:10.1111/fcp.12049.
[42] M. Kozłowska, E. Gruczyńska, I. Ścibisz, M. Rudzińska, Fatty acids and sterols composition, and antioxidant activity of oils extracted from plant seeds, Food Chem. 213 (2016) 450–456, https://doi.org/10.1016/j.foodchem.2016.06.102.
[43] D. Zhao, J.T. Kuethe, M. Journet, Z. Peng, G.R. Humphrey, Efficient and practical synthesis of (R) -2-Methylpyrrolidine, J. Org. Chem. 71 (2006) 4336–4338.
[44] M. Ganesh, M. Mohankumar, Extraction and identification of bioactive components in Sida cordata (Burm. f.) using gas chromatography – mass spectrometry, J. Food Sci. Technol. 54 (2017) 3082–3091. doi:10.1007/s13197-017-2744-z.
[45] M. Couto, C. Barbosa, D. Silva, A. Rudnitskaya, L. Delgado, Oxidative stress in asthmatic and non-asthmatic adolescent swimmers - a breathomics approach, Pediatr. Allergy Immunol. (2017) 452–457, https://doi.org/10.1111/ijlh.12426.
[46] M. Singh, J. Smith, M. Bailey, Using natural antimicrobials to enhance the safety and quality of poultry, Elsevier Ltd (2015), https://doi.org/10.1016/B978-1-78242-034-7.00018-9.
[47] R. De Cássia, L.N. Andrade, D.P. De Sousa, A review on anti-inflammatory activity of monoterpenes, Molecules 18 (2013) 1227–1254, https://doi.org/10.3390/molecules18011227.
[48] E. Liaw, K. Liu, Synthesis of terpinyl acetate by lipase-catalyzed esterification in supercritical carbon dioxide, Bioresour. Technol. 101 (2010) 3320–3324, https://doi.org/10.1016/j.biortech.2009.11.081.
[49] J. Hosoi, M. Tanida, T. Tsuchiya, Mitigation of stress-induced suppression of contact hypersensitivity by odorant inhalation, Br. J. Dermatol. 145 (2001) 716–719.
[50] D. Wang, C. Wang, X.I.N. Pi, L.E.I. Guo, Y.U.E. Wang, M. Li, Y.U.E. Feng, Z. Lin, W. E.I. Hou, E. Li, Urinary volatile organic compounds as potential biomarkers for renal cell carcinoma, Biomed. Rep. 5 (2016) 68–72, https://doi.org/10.3892/br.2016.686.
[51] C. Sarikurkcu, M.S. Ozer, N. Calli, J. Popović-Djordjević, Essential oil composition and antioxidant activity of endemic Marrubium parviflorum subsp. oligodon, Ind. Crops Prod. 119 (2018) 209–213, https://doi.org/10.1016/j.indcrop.2018.04.023.
[52] B. Uma, R. Parvathavarthini, Antibacterial effect of hexane extract of sea Urchin, Temnopleurus alexandri (Bell 1884), Int. J. PharmTech Res. 2 (2010) 1677–1680.
[53] M. Ram, K. Rao, The GC-MS study of one ayurvedic preparation Katakakhadiradi Kashayam, Int. J. Pharm. Sci. Rev. Res. 39 (2016) 216–224.
[54] A.G. Pirbalouti, H. Mirbagheri, B. Hamedi, E. Rahimi, Antibacterial activity of the essential oils of myrtle leaves against Erysipelothrix rhusiopathiae, Asian Pac. J. Trop. Biomed. 4 (2014) S505–S509, https://doi.org/10.12980/APJTB.4.2014B1168.
[55] W. Okiei, M. Ogunlesi, E. Ofor, E.A.S. Osibote, Analysis of essential oil constituents in hydro-distillates of calotropis procera (Ait.) R.Br, Res. J. Phytochem. 3 (2009) 44–53.
[56] W. Lee, E.R. Woo, D.G. Lee, Phytol has antibacterial property by inducing oxidative stress response in Pseudomonas aeruginosa, Free Radic. Res. 50 (2016) 1309–1318, https://doi.org/10.1080/10715762.2016.1241395.
[57] F. Mericli, E. Becer, H. Kabadayı, A. Hanoglu, D.Y. Hanoglu, D.O. Yavuz, T. Ozek, S. Vatansever, Fatty acid composition and anticancer activity in colon carcinoma cell lines of Prunus dulcis seed oil, Pharm. Biol. 55 (2017) 1239–1248, https://doi.org/10.1080/13880209.2017.1296003.
[58] Y. Wang, Y. Qian, Q. Fang, P. Zhong, W. Li, L. Wang, W. Fu, Y. Zhang, Z. Xu, X. Li, G. Liang, Saturated palmitic acid induces myocardial inflammatory injuries through direct binding to TLR4 accessory protein MD2, Nat. Publ. Gr. 8 (2018) 1–13, https://doi.org/10.1038/ncomms13997.
[59] M. Mcguire, R. Li, R. Wang, F. Yu, Isolation and characterization of methyl esters and derivatives from Euphorbia kansui and their inhibitory effects on the human, J. Pharm. Pharm. Sci. 8 (2005) 528–535.
[60] S.L. Mumford, J.E. Chavarro, C. Zhang, N.J. Perkins, L.A. Sjaarda, A.Z. Pollack, K.C. Schliep, K.A. Michels, S.M. Zarek, T.C. Plowden, R.G. Radin, L.C. Messer, R.A. Frankel, J. Wactawski-wende, Dietary fat intake and reproductive hormone concentrations and ovulation in regularly menstruating women, Am. J. Clin. Nutr. (2016) 1–10, https://doi.org/10.3945/ajcn.115.119321.
[61] M.A. Belury, R.M. Cole, D.B. Snoke, T. Banh, A. Angelotti, Linoleic acid, glycemic control and Type 2 diabetes, Prostaglandins, Leukot. Essent. Fat. Acids. 132 (2018) 30–33, https://doi.org/10.1016/J.PLEFA.2018.03.001.
[62] S. Arslan, Antimicrobial activity studies on some piperidine and pyrrolidine substituted halogenobenzene derivatives, J. Enzyme Inhib. Med. Chem. 21 (2006) 211–214, https://doi.org/10.1080/14756360600563063.
[63] H.M. Dodds, M. Findlay, J. Robert, R. Prince, A. Hospital, M. Findlay, Clinical pharmacokinetics of the irinotecan metabolite 4-piperidinopiperidine and its possible clinical importance, Cancer Chemother Pharmacol. 45 (2000) 9–14.
[64] C. Morera, S. Sabates, A. Jaen, Sex differences in N -palmitoylethanolamide effectiveness in neuropathic pain associated with lumbosciatalgia, Pain Manag. 5 (2015) 81–87, https://doi.org/10.2217/pmt.15.5.
[65] C. Saturnino, A. Popolo, A. Ramunno, S. Adesso, M. Pecoraro, M.R. Plutino, S. Rizzato, A. Albinati, S. Marzocco, M. Sala, D. Iacopetta, M.S. Sinicropi, Anti-inflammatory, antioxidant and crystallographic studies of N-Palmitoyl-ethanol amine (PEA) derivatives, Molecules 22 (2017) 1–11, https://doi.org/10.3390/molecules22040616.
[66] Y.V. Polonskaya, V.S. Shramko, S.V. Morozov, E.I. Chernyak, A.M. Chernyavsky, Y.I. Ragino, Balance of fatty acids and their correlations with parameters of lipid metabolism and markers of inflammation in men with coronary atherosclerosis, Bull. Exp. Biol. Med. 164 (2017) 33–35, https://doi.org/10.1007/s10517-017-3920-x.
[67] M. Nadeem, M. Imran, I. Taj, M. Ajmal, M. Junaid, Omega-3 fatty acids, phenolic compounds and antioxidant characteristics of chia oil supplemented margarine, Lipids Health Dis. 16 (2017) 1–12, https://doi.org/10.1186/s12944-017-0490-x.
[68] U.N. Das, Essential fatty acids enhance free radical generation and lipid peroxidation to induce apoptosis of tumor cells, Clin. Lipidol. 6 (2011) 463–489, https://doi.org/10.2217/clp.11.34.
[69] N. Gotoh, Y. Noguchi, A. Ishihara, K. Yamaguchi, H. Mizobe, T. Nagai, I. Otake, K. Ichioka, S. Wada, Highly unsaturated fatty acid might act as an antioxidant in emulsion system oxidized by azo compound, J. Oleo Sci. 59 (2010) 631–639, https://doi.org/10.5650/jos.59.631.
[70] P. Swords, A. Van, Ryckeghem, Summary of commercially available pheromones of common stored-product moths, 10th Int. Work. Conf. Stored Prod. Prot. (2010) 1008–1010, https://doi.org/10.5073/jka.2010.425.491.
[71] A.R. Chabukswar, B.S. Kuchekar, S.C. Jagdale, P.D. Lokhande, V.V. Chabukswar, S.U. Shisodia, R.H. Mahabal, A.M. Londhe, N.S. Ojha, Synthesis and evaluation of analgesic, anti-asthmatic activity of (E)-1-(8-hydroxyquinolin-7-yl)-3-phenylprop-2-en-1 ones Synthesis, evaluation, analgesic anti-asthmatic activity of (E)-1-(8-hydroxyquinolin-7-yl)-3-phenylprop-2-en-1 one, Arab. J. Chem. 9 (2016) 704–712, https://doi.org/10.1016/j.arabjc.2014.10.046.
[72] E. Ramesh, R.D.R.S. Manian, R. Raghunathan, S. Sainath, M. Raghunathan, Synthesis and antibacterial property of quinolines with potent DNA gyrase activity, Bioorganic Med. Chem. 17 (2009) 660–666, https://doi.org/10.1016/j.bmc.2008.11.058.
[73] M. Abubakar, R. Majinda, GC-MS analysis and preliminary antimicrobial activity of Albizia adianthifolia (Schumach) and Pterocarpus angolensis (DC), Medicines. 3 (2016) 3, https://doi.org/10.3390/medicines3010003.
[74] Y. Li, D. Kong, H. Wu, Analysis and evaluation of essential oil components of cinnamon barks using GC – MS and FTIR spectroscopy, Ind. Crop. Prod. 41 (2013) 269–278, https://doi.org/10.1016/j.indcrop.2012.04.056.
[75] N. Arora, S. Pandey-rai, GC – MS analysis of the essential oil of Celastrus paniculatus Willd. seeds and antioxidant, anti-inflammatory study of its various solvent extracts, Ind. Crop. Prod. 61 (2014) 345–351, https://doi.org/10.1016/j.indcrop.2014.07.025.
[76] K.J. Lee, Y.C. Oh, W.K. Cho, J.Y. Ma, Antioxidant and anti-inflammatory activity determination of one uundred kinds of pure chemical compounds using offline and online screening HPLC assay, Evidence-Based Complement. Altern. Med. 2015 (2015) 1–13, https://doi.org/10.1155/2015/165457.
[77] A. Floegel, D. Kim, S. Chung, S.I. Koo, O.K. Chun, Comparison of ABTS/DPPH assays to measure antioxidant capacity in popular antioxidant-rich US foods, J. Food Compos. Anal. 24 (2011) 1043–1048, https://doi.org/10.1016/j.jfca.2011.01.008.
[78] N. Semmar, M. Farman, J.S.O. Mccullagh, Measurement of total phenolic content and antioxidant activity of aerial parts of medicinal plant Coronopus didymus, Asian Pac. J. Trop. Med. 10 (2017) 792–801, https://doi.org/10.1016/j.apjtm.2017.07.024.