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# Assessment of Antioxidant, Antibacterial Activity and Phytoactive Compounds of Aqueous Extracts of Avocado Fruit Peel from Ethiopia

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## Abstract

The present study is focused to assess the efficiency of avocado fruit peel waste in different in vitro activities in order to explore the possibility of utilizing waste as a value-added product in various applications. Preliminary phytochemical screening confirmed the presence of alkaloids, flavonoids, phenols, tannins, glycosides, and absence of sterols, saponins, resins, and thiols. Remarkable free radicals scavenging ability was observed in all the tested radicals namely, DPPH·($IC_{50}$ = 71.96 ± 0.44 µg/ml), nitric oxide (NO·) ($IC_{50}$ = 149.46 µg/ml), hydroxyl (·OH) (107.91 ± 3.59 µg/ml), Superoxide ($O_2^{\cdot-}$) ($IC_{50}$ = 103.05 ± 2.19 µg/ml). In the antibacterial assay, the zone of inhibition was recorded as 9.5 ± 0.5, 12.0 ± 1.21, 7.5 ± 0.35, 6.0 ± 0.5 and 10.0 ± 1.0 for the strains of *Escherichia coli*, *Klebsiella pneumonia*, *Pseudomonas* spp., *Staphylococcus aureus,* and *Bacillus* spp. respectively. The Fourier Transform Infrared (FTIR) profiling indicated the existence of resilient functional groups and the gas chromatography–mass spectrometry (GC–MS) profiling was exhibited the presence of eighteen major components highly accountable for its pharmaceutical activities.

**Keywords** Therapeutic · Antagonistic · Antioxidant · Functional groups · Waste conversion

## Introduction

Avocado (also addressed as 'Alligator pear') is a significantly nourishing fruit with high nutritional value and it is regarded as a major tropical fruit. It contains a high source of protein, carbohydrate, fiber and essential micronutrients such as minerals, vitamins, and polyphenols (Pennington and Fisher 2009; Rotta et al. 2016). The volume of fruit yield is high in avocado trees and it produces a yield of 138 kg at seven year span after planting. The avocado (*Persea americana* Mill.) is a perennial plant cultivated on rough locations and does not compete with the annuals adopted to flatlands (Bleinroth and Castro 1992; Duarte et al. 2016). Studies report that there is a very less knowledge among farmers about the nutritional as well as value-added elements present in vegetable and fruit waste; Thus the residual waste accumulating in tons are not harnessed or channeled for other valuable purposes (Rotta et al. 2016). Along the same line, avocado wastages can be very well harnessed, and recently avocado tree has attracted much interest among farmers as it is one of the most important productive plants per unit of the cultivated field (Vinha et al. 2013). The non-edible parts of the fruit (peel and seed) were also analyzed in order to evaluate their possible role as an inexpensive source of precursor/substrate for several applications. The utilization of non-edible parts of the fruit is under subject to study which may prove to be very lucrative for the farmers in the imminent. Because primarily it entails an imperative reduction in the generation of waste and also non-edible parts of certain fruits can be a source of high levels of valuable bioactive compounds.

Phytotherapy is the oldest form of health care known to mankind and bioactive substances present in plants are well-known for their antimicrobial, antioxidant and immunomodulatory properties. Previous studies report that more

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than 400,000 species of tropical flowering plants possess medicinal properties and that is the reason for traditional medicine to be cheaper than modern medicine, particularly in the developing countries (Uthayakumar et al. 2014). Profiling of bioactive metabolites from the plants delivers valuable information about their chemical diversities, toxicity concerns and medicinal potentials that are relevant to various fields (Ezekwe and Chikezie 2017). Green extraction processes are considered as an important tool in discovering and extracting phytochemicals possessing beneficial properties with non-toxic nature (Tremocoldi et al. 2018). GC–MS offers a reliable and reproducible analytical protocol for the profiling of the bioactive principles from the extracts among other hyphenated techniques described elsewhere (Ezekwe and Chikezie 2017).

Thus, there is a need to investigate the efficiency of avocado fruit peel waste as an abundant and/or an inexpensive source for therapeutic applications. Therefore, the present work is aimed to assess the avocado fruit peel aqueous extracts in terms of their preliminary phytochemical testing, antioxidant and antibacterial efficiency. Furthermore, the phytoactive compounds responsible for these activities were also analyzed.

## Materials and Methods

### Plant Material Collection and Processing

Avocado fruit peel (AFP) waste was collected from the local market at the premises of Addis Ababa Science and Technology University, Ethiopia. Avocado fruit peel powder (AFPP) was prepared from dried fruit peel material. AFP aqueous extract (AFPAE) was prepared from 20 g of dry AFPP with 1 L distilled water and heated at 80 °C for 20 min on a hot plate. This solution was filtered by filter paper (Whatman No 42, Maidstone, England) and stored at 4 °C for further experiments.

### Phytochemical Analysis

#### Qualitative Phytochemical Analysis

The phytochemical screening of AFPAE is assayed by standard methods (Harborne and Trease 1978). The screening was carried out to discover the significant bioactive components such as tannins, saponins, flavonoids, phenols, terpenoids, alkaloids, glycosides, cardiac glycosides, coumarins, and steroids.

#### Quantitative Phytochemical Analysis

**Determination of Total Phenol Content** The total phenolic content of AFPAE was determined according to the method described by Siddhuraju and Becker (2003).

**Determination of Total Tannin Content** Tannins were estimated after treatment with polyvinylpolypyrrolidone (PVPP) according to Siddhuraju and Manian (2007).

**Determination of Total Flavonoid Content** The flavonoid content was determined by the use of a slightly modified colorimetry method described previously by Zhishen et al. (1999).

### In Vitro Antioxidant Activities

Different concentrations (50–250 µg/ml) of AFPAE were tested for various types of radicals scavenging potential. Ascorbic acid was used as standard reference compounds for all in vitro antioxidant assays.

#### Free Radical Scavenging Activity on DPPH·

The antioxidant activity of the sample was determined in terms of hydrogen donating or radical scavenging ability, using the stable radical DPPH, according to the method of Blois (1958).

#### Reducing Power Assay

The reducing power of the sample extract was determined by the method reported by Siddhuraju et al. (2002).

#### Hydroxyl Radical Scavenging Activity

The scavenging activity of the sample on hydroxyl radical was measured according to the method of Klein et al. (1991).

#### Superoxide Radical Scavenging Activity

Superoxide radicals were generated by a modified method of Beauchamp and Fridovich (1971).

#### Nitric Oxide Radical Scavenging Activity

The nitric oxide scavenging activity of the sample was measured according to the method of Sreejayan and Rao (1997).

#### Ferric Reducing Antioxidant Power (FRAP) Assay

The FRAP assay was used to estimate the reducing capacity of the sample, according to the method of Benzie and Strain (1996).

### In Vitro Antibacterial Activity of AFPAE

The antibacterial efficacy of the AFPAE (30 µl) was tested against the organism like *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumonia*, *Pseudomonas* spp. and *Bacillus* spp. by disc diffusion according to Rodriguez-Carpena et al. (2011) with minor changes. A 30 µl of sterile distilled water and standard antibiotic (Tetracyclin-20 µg/ml) were loaded in separate disc denoting the negative and positive control respectively.

### Fourier Transform Infrared (FTIR) and Gas Chromatography–Mass Spectrometry (GC–MS) Analysis

FTIR (Model Avatar, 370 Spectrometer, Thermo Nicolet Corporation, Madison, USA) was used to analyze the functional group presents on the AFPAE in comparison with AFPP. The main phytocomponents of AFPAE were identified by using GC–MS detection system. The samples were suspended with ethanol and subjected to GC–MS analysis. Elucidation of phytocomponents was assayed by comparison of their retention times and mass with their regular authentic standard spectra using computer searches in NIST08.L and Wiley7n.l libraries (Ezekwe and Chikezie 2017).

**Table 1** Qualitative phytochemical screening of AFPAE

| S. No | Test | Inference [presence (+); absence (−)] |
|---|---|---|
| 1 | Alkaloids | + |
| 2 | Flavanoids | + |
| 3 | Saponins | − |
| 4 | Phenols | + |
| 5 | Sterols | − |
| 6 | Glycosides | + |
| 7 | Resins | − |
| 8 | Thiols | − |
| 9 | Tannins | + |

**Table 2** Total phenol, tannin and flavonoid content of AFPAE

| Phytochemicals | Content |
|---|---|
| Total phenol | 51.58 ± 2.02 (mg GAE/g extract) |
| Tannin | 4.33 ± 0.64 (mg TAE/g extract) |
| Flavonoid | 2.25 ± 0.02 (mg RE/g extract) |

*GAE* gallic acid equivalent, *TAE* tannic acid equivalent, *RE* rutin equivalent, values are expressed as mean ± SD of triplicates

## Results and Discussion

### Phytochemical Screening

Phytochemical screening of AFPAE confirmed the presence of alkaloids, flavonoids, phenols, glycosides, and tannins in different qualitative ranges. The negative sign depicted the absence of sterols, saponins, resins, and thiols (Table 1).

Among the nine phytoconstituents, phenol, flavonoid, and tannin showed a strong presence depicting the pharmaceutical property of the avocado peel extracts. Hence all these secondary metabolites in the extracts were quantified further to assay the best extraction possessing the significant levels of bioactive components (Table 2).

Total phenolics content in the AFPAE was 51.58 ± 2.02 mg of Gallic Acid equivalents (GAE)/g. Whereas, total tannin content in the AFPAE was 4.33 ± 0.64 mg of Tannic acid equivalents (TAE)/g. Total flavonoid content in the AFPAE was 2.25 ± 0.02 mg of Rutin equivalent (RE)/g (Table 2). Suganyadevi et al. (2011) have reported that phenolic compounds contain an enhanced anti-oxidative property which could suppress the initiation or propagation of chain reactions. The antioxidant ability of phenolic compounds is mainly due to their redox properties, which permits them to act as reducing agents, hydrogen donors and quenchers of singlet oxygen and thus plays a vital role in free radical scavenging. Rama et al. (2013) have reported that polyphenols have attracted considerable attention due to their physiological functions, including antioxidant, anti-mutagenic, and antitumor activities which have beneficial implications for improving health.

Tannin levels were predominantly found high in AFPAE showing the better extraction properties of water as a solvent (Table 2). Prasad et al. (2008) have reported that tannins possess significant antimicrobial agents and contains water-soluble polyphenols and exist as precipitated proteins in many plant foods. It is also found to prevent the growth of microorganisms by precipitating the microbial proteins and also inhibits the growth of many fungi, yeasts, bacteria, and viruses. Gulcin et al. (2010) have reported that high levels of tannins are responsible for free radical scavenging and antioxidant efficacy.

In the present analysis, the levels of flavonoids were considerably high in AFPAE elucidating the importance of the extract as a potent medicinal agent. Flavonoids are known to be synthesized by plants as a reflection to microbial infection. Hence it should not be surprising that they act as profound antibacterial substances against a wide range of infectious agents (Jasmine et al. 2007). Tapas et al. (2008) have stated that flavonoids possess significant nutraceuticals, antimicrobial and antioxidant activity.

## Antioxidant Activity

In the present study, AFPAE was analyzed for an anti-oxidant activity via antioxidant assays (Table 3). The AFPAE, when subjected to DPPH·assay, showed maximum free radical scavenging activity of 60.13 ± 0.79% at a concentration of 250 µg when compared with standard ascorbic acid levels, thus elucidating the importance of the AFPAE as a potent anti-oxidant agent (Table 3). Bright and Kanagappan (2016) have reported the presence of significant levels of antioxidants in five different aquatic weeds of India and revealed the role of aquatic weeds in disease prevention and the credit has been contributed to the antioxidant properties of the plant phytoconstituents. It is believed that a higher intake of antioxidant-rich food is related to a decreased risk of degenerative diseases, particularly enhanced protection from a broad range of infectious agents and cancer causative agents.

The hydroxyl radical is the most reactive free radical formed in the biological fluids. It has been understood as a major active oxygen-centered radical formed from the reaction of various hydroperoxides with transition metal ions, which has the ability to damage almost every molecule found in the living system causing lipid peroxidation and biological damage (Engwa 2018). The maximum percentage inhibition of AFPAE was found to be 31.87 ± 1.39% at a concentration of 250 µg with an IC 50 value of 107.91 ± 3.59 (µg/ml) (Table 3).

Nitric oxide is a free radical component generated by endothelial cells, macrophages, and neuron, etc., and involved in the modulation and regulation of various physiological processes (Rao et al. 2013). Excess concentration is connected with the onset of several diseases. It reacts with oxygen to produce its stable products of nitrate and nitrite through intermediates $NO_2$, $N_2O_4$, and $N_3O_3$.In the present analysis, nitric oxide radical scavenging ability of AFPAE was found to be 48.54 ± 1.08% at a concentration of 250 µg with an IC 50 value of 79.05 ± 0.34 (µg/ml) (Table 3).

Superoxide anion ($O_2^{\cdot -}$) is an extremely reactive compound synthesized when oxygen is reduced by a single electron and may be produced during the regular catalytic role of various enzymes. Studies report that superoxide anion is highly harmful to cellular components. Robak and Glyglewski (1988) studied that flavonoids are the most effective antioxidants because they scavenge a large range of superoxide anions. In the present study, the superoxide radical scavenging activities of the plant extract had markedly increased with concentrations. The results indicate that the radical scavenging ability of AFPAE was found to be 36.99 ± 1.17% at a concentration of 250 µg with an IC 50 value of 103.05 ± 2.19 (µg/ml) (Table 3).

In the present study, the absorbance at 700 nm increased from 0.09 ± 0.002 (50 µg) to 0.24 ± 0.005 (250 µg) (Table 4),

**Table 3** In vitro antioxidant activity of AFPAE

| Anti-oxidant assay | Sample concentration (µg/reaction volume) | Inhibition (%) | IC50 (µg/ml) |
|---|---|---|---|
| DPPH radical scavenging activity | 50 | 24.78 ± 0.51 | 71.96 ± 0.44 |
| | 100 | 28.76 ± 0.92 | |
| | 150 | 40.97 ± 0.99 | |
| | 200 | 47.13 ± 0.87 | |
| | 250 | 60.13 ± 0.79 | |
| Hydroxyl radical scavenging activity | 50 | 8.17 ± 0.99 | 107.91 ± 3.59 |
| | 100 | 12.91 ± 1.49 | |
| | 150 | 19.10 ± 0.99 | |
| | 200 | 22.66 ± 0.46 | |
| | 250 | 31.87 ± 1.39 | |
| Nitric oxide radical scavenging activity | 50 | 31.15 ± 0.85 | 79.05 ± 0.34 |
| | 100 | 35.38 ± 0.92 | |
| | 150 | 39.02 ± 0.77 | |
| | 200 | 42.37 ± 0.92 | |
| | 250 | 48.54 ± 1.08 | |
| Super oxide radical scavenging activity | 50 | 12.97 ± 0.84 | 103.05 ± 2.19 |
| | 100 | 15.97 ± 0.99 | |
| | 150 | 21.84 ± 1.23 | |
| | 200 | 28.82 ± 1.12 | |
| | 250 | 36.99 ± 1.17 | |

Values are expressed as mean ± SD of triplicates

which shows the FRAP activity of AFPAE was concentration-dependent. FRAP value serves as a measure of Fe (II) TPTZ reducing the power of the extract. Reducing power of a compound is related to its electron transfer ability and it's mainly used to assess the antioxidant ability of polyphenols, which is related to the presence of reductones, which exhibits antioxidant activity by interrupting the free radical chain by donating a hydrogen atom (Duan et al. 2007).

## In Vitro Antibacterial Activity of AFPAE

Inhibition zone diameters exerted in disc diffusion method was recorded as evidence for the antimicrobial nature of AFPAE against screened strains. In general, a diverse range of antimicrobial activity of AFPAE was observed against all the bacteria tested in this study and significant variance were recorded among Gram-positive bacteria (*Staphylococcus* spp. and *Bacillus* spp.) were generally known to be more sensitive than Gram-negative bacteria (*E. coli, Klebsiella* spp. and *Pseudomonas* spp.) (Table 5). More intense effect of plant/fruit extracts against Gram-positive strains than Gram-negative was reported in previous studies (Bamoniri et al. 2010). Even though the Gram-negative bacteria have an extra productive outer membrane, which accepts some drugs and antibiotics from penetrating the cell, partially accounting to more resistant to antibiotics than Gram-positive bacteria (Kossah et al. 2011; Rodríguez-Carpena et al. 2011). The highest inhibitory effect was observed as 12.0 ± 1.21 mm for *Staphylococcus* spp. in Gram-positive group and 9.5 ± 0.5 for *E. coli* in the Gram-negative group tested. *Pseudomonas* spp. was identified as the most resistant bacteria (6.0 ± 0.5 mm) from the group of bacteria screened in this report (Table 5). The antimicrobial activity of AFPAE on test microorganism could be due to the nature of the antimicrobial matters presented in the extracts and their mode of mechanisms (Shan et al. 2007).

## FTIR Analysis

The FTIR spectra (Fig. 1) showed significant reduction of peaks between AFPP and AFPAE. It is evident that the majority of functional groups is extracted from AFPP by the aqueous solvent in the extraction process. Figure 1. exhibited the peaks at the regions of 1253 $cm^{-1}$, 1623 $cm^{-1}$ and 2911 $cm^{-1}$ corresponding to the functional groups (compounds indicated) of –c–CO–c stretching (aromatics), C=C stretching (Alkenes) and C=H stretching (Alkanes) respectively. The peak at 1758 $cm^{-1}$ corresponds to the C=O bond stretching indicating the presence of phytochemicals in the plant extract and amino acids (Kanagasubbulakshmi and Kadirvelu 2017). The broad peak observed at 3443 $cm^{-1}$ corresponds to polyphenols. Bands at 602 $cm^{-1}$, 1028 $cm^{-1}$, 1409 $cm^{-1}$, 1640 $cm^{-1}$, 2361 $cm^{-1}$, and 2938 $cm^{-1}$ indicate the bioactive compounds such as alkyl halides, alkanes, sulfonamides, sulfonyl chlorides and ester (Kavipriya and Chandran 2018). The FTIR results indicated the existence of resilient functional groups in the AFPAE, which act as better agents for multiple biological applications.

**Table 4** Reducing power activity of AFPAE

| Sample concentration (µg/reaction volume) | Absorbance at 700 nm |
|---|---|
| 50 | 0.09 ± 0.002 |
| 100 | 0.13 ± 0.006 |
| 150 | 0.16 ± 0.003 |
| 200 | 0.21 ± 0.002 |
| 250 | 0.24 ± 0.005 |

Values are expressed as mean ± SD of triplicates

**Table 5** In vitro antibacterial activity of AFPAE

| Microbial culture | Zone of inhibition (mm) | | |
|---|---|---|---|
| | AFPAE | Tetracyclin | Sterile distilled water |
| *Escherichia coli* | 9.5 ± 0.5 | 20 ± 1.5 | No zone of inhibition |
| *Staphylococcus aureus* | 12.0 ± 1.21 | 23 ± 1.0 | |
| *Klebsiella pneumonia* | 7.5 ± 0.35 | 11 ± 1.0 | |
| *Pseudomonas* spp. | 6.0 ± 0.5 | 17 ± 1.0 | |
| *Bacillus* spp. | 10.0 ± 1.0 | 20 ± 1.2 | |

Values are expressed as mean ± SD of triplicates

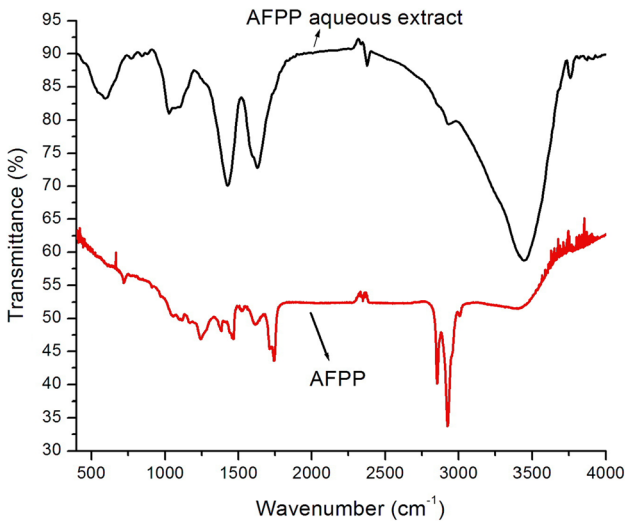



**Fig. 1** FTIR spectra of AFPP and its aqueous extract

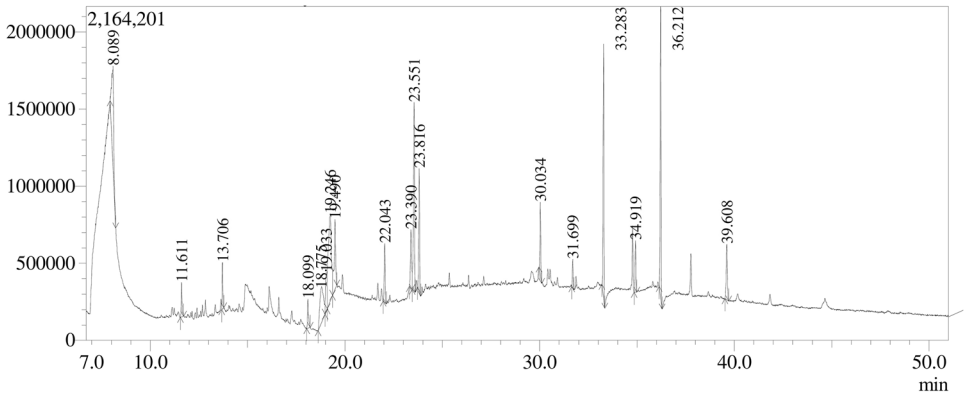



**Fig. 2** GC–MS chromatogram of AFPAE

## GC–MS Analysis

GC–MS results of AFPAE confirmed the presence of eighteen major components (Fig. 2) highly responsible for its antioxidant, antibacterial and antioxidant properties. The active biomolecules and their retention time (RT), peak area, molecular formula, molecular weight (MW) and structures obtained from PubChem sources are presented in Table 6. The first compound identified with less RT (8.08 min) was assigned as glycerin and the compound which took long RT (39.60 min) was identified as pentacosane. Specifically, hexadecanoic acid and its derivative compounds possess a high percentage of peak area among the entire phytocompounds present in AFPAE (Fig. 2, Table 6). The compounds elucidated in GC–MS spectra are profoundly known for their therapeutical properties.

The pharmaceutical role of hexadecanoic acid is more evident from previous studies. Abubakar and Majinda (2016) have reported the antimicrobial efficacy of hexadecanoic acid activity against Gram-positive and Gram-negative microorganisms. Aparna et al. (2012) have assessed the anti-inflammatory property of hexadecanoic acid and their role in medicinal oils in treating chronic diseases. More importantly, the significant antioxidant role of hexadecanoic acid is reported by Patra et al. (2015).

The presence of octadecenoic acid (z)-, methyl ester was observed in this study at 23.55 min RT with the peak area of 9.79% (Fig. 2, Table 6). Previous studies reported that the octadecanoic acid and its closely related compounds such as 9-octadecenoic acid (z)-, methyl ester from different plant extracts are possessing anti-inflammatory, anticancer and antiandrogenic activity (Manonmani and Catharin 2015).

## Conclusion

The present investigation revealed that AFPAE was comprised of a variety of metabolites which possess strong antioxidant activities. The AFPAE was studied to possess better antibacterial efficacy against the selective bacterial cultures. Existence of resilient functional groups in the AFPAE is profiled by FTIR analysis. Wherein, GC–MS analysis revealed the presence of 18 major components in AFPAE. These substances could be isolated and empirically evaluated further to confirm their biologic and medicinal activities. However, a detailed study is needed to elucidate the mechanism of chemical constituents available in AFPAE for their better utility in various applications.

**Table 6** Phytocomponents of AFPAE detected by GC–MS

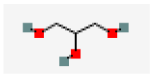
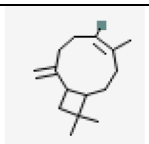
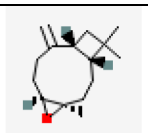
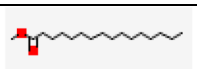
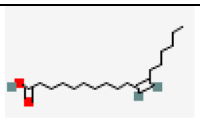
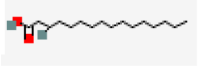
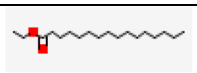
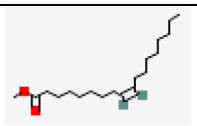
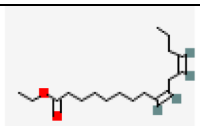
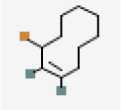
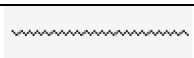
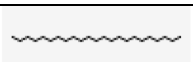
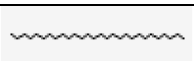
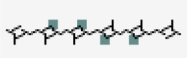
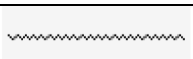
| Peak no. | Retention time ($t_R$) (Min) | Area % | Compounds | Molecular formula | Molecular weight (g/mol) | 2D structure (Source: PubMed) | PubChem CID |
|---|---|---|---|---|---|---|---|
| 1 | 8.089 | 10.56 | Glycerin | $C_3H_8O_3$ or $CH_2OH–CHOH–CH_2OH$ | 92 |  | 753 |
| 2 | 11.611 | 1.49 | Caryophyllene | $C_{15}H_{24}$ | 204.357 |  | 5322111 |
| 3 | 13.706 | 1.53 | (−) −Beta caryophyllene epoxide | $C_{15}H_{24}O$ | 220.356 |  | 6604672 |
| 4 | 18.099 | 1.63 | Hexadecanoic acid, methyl ester | $C_{17}H_{34}O_2$ | 270.457 |  | 8181 |
| 5 | 18.775 | 6.96 | Cis-vaccenic acid | $C_{18}H_{34}O_2$ | 282.468 |  | 5282761 |
| 6 | 19.033 | 3.10 | Ethyl 9-hexadecenoate | $C_{18}H_{34}O_2$ | 282.468 |  | 5364759 |
| 7 | 19.246 | 14.66 | Hexadecanoic acid | $C_{16}H_{32}O_2$ | 256.43 |  | 985 |
| 8 | 19.490 | 5.79 | Hexadecanoic acid, ethyl ester | $C_{18}H_{36}O_2$ | 284.484 |  | 12366 |
| 9 | 22.043 | 2.82 | 9-Octadecenoic acid (Z)-, methyl ester | $C_{19}H_{36}O_2$ | 296.495 |  | 5364509 |
| 10 | 23.390 | 3.59 | Ethyl 9,12-hexadecadienoate | $C_{18}H_{32}O_2$ | 280.452 |  | 91694367 |

**Table 6** (continued)

| Peak no. | Retention time ($t_R$) (Min) | Area % | Compounds | Molecular formula | Molecular weight (g/mol) | 2D structure (Source: PubMed) | PubChem CID |
|---|---|---|---|---|---|---|---|
| 11 | 23.551 | 9.79 | (E)-9-Octadecenoic acid ethyl ester | $C_{20}H_{38}O_2$ | 310.522 |  | 5364430 |
| 12 | 23.816 | 5.52 | Cyclodecene, 3-bromo- | $C_{10}H_{17}Br$ | 217.15 |  | 5367605 |
| 13 | 30.034 | 2.90 | Tetratetracontane | $C_{44}H_{90}$ | 619.204 |  | 23494 |
| 14 | 31.699 | 1.08 | Tricosane | $C_{23}H_{48}$ | 324.637 |  | 12534 |
| 15 | 33.283 | 10.48 | Octacosane | $C_{28}H_{58}$ | 394.772 |  | 12408 |
| 16 | 34.919 | 2.17 | Squalene | $C_{30}H_{50}$ | 410.73 |  | 638072 |
| 17 | 36.212 | 12.36 | Tetratetracontane | $C_{44}H_{90}$ | 619.204 |  | 23494 |
| 18 | 39.608 | 3.57 | Pentacosane | $C_{25}H_{52}$ | 352.691 |  | 12406 |

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## Compliance with Ethical Standards

**Conflict of interest** The authors did not declare any conflict of interest.

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