

## Phytochemical and Gas Chromatography–Mass Spectrometry (GC–MS) Analysis of Avocado Pear (*Persea americana*) Seeds

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### ABSTRACT

Avocado pear (*Persea americana*) seed is an underutilised plant by-product with considerable potential as a source of bioactive compounds. This study evaluated the phytochemical composition and chemical constituents of avocado seed extract using quantitative phytochemical analysis and Gas Chromatography–Mass Spectrometry (GC–MS). Mature avocado fruits were obtained from Etche markets in Rivers State, Nigeria. The seeds were removed, washed, air-dried, pulverised and extracted using Soxhlet extraction with aqueous and ethanolic solvents. Quantitative analysis revealed the presence of eight phytochemical constituents, with phenols having the highest concentration (28.11 mg/100 g), followed by flavonoids (18.54 mg/100 g), tannins (9.08 mg/100 g), glycosides (6.19 mg/100 g), steroids (3.47 mg/100 g), saponins (2.15 mg/100 g) and terpenes (1.67 mg/100 g), while alkaloids recorded 3.90%. GC–MS analysis identified 26 chemical constituents, including furfural, phytol, 5-hydroxymethylfurfural,  $\beta$ -sitosterol, quercetin, stigmaterol, campesterol, gallic acid, oleic acid, catechin, epicatechin, squalene,  $\alpha$ -amyrin,  $\beta$ -amyrin, lupeol, linalool, geraniol,  $\beta$ -caryophyllene, dodecanoic acid and linoleic acid. Linoleic acid recorded the highest peak area (13.78%), followed by Oleic acid (10.92%) and Hexadecanoic acid methyl ester (9.78%). The findings demonstrate that avocado seed contains diverse phytochemical and chemically characterised constituents of potential biological significance, supporting its further investigation as a source of bioactive compounds.

**Keywords:** Avocado Seed, Gas Chromatography–Mass Spectrometry, Soxhlet Extraction, Phytochemical Constituents.

### Introduction

Avocado (*Persea americana* Mill.) is an economically important fruit crop belonging to the family Lauraceae and is widely cultivated in tropical and subtropical regions. The fruit is recognized for its nutritional value and contains lipids, proteins, carbohydrates, vitamins, minerals and a wide range of phytochemicals with potential biological activities (Dreher & Davenport, 2013 Araújo et al., 2021). Increasing global avocado production and consumption has also resulted in the generation of substantial quantities of agro-industrial residues, particularly peels and seeds, which are commonly discarded (Dabas et al., 2013; Araújo et al., 2021; Rodríguez-Carpena et al., 2021). The avocado seed represents a considerable proportion of the fruit and has traditionally received less attention than the edible pulp.

However, studies have established that the seed contains diverse bioactive constituents, including phenolic compounds, flavonoids, procyanidins, catechins and other secondary metabolites (Dabas et al., 2013; Rodríguez-Carpena et al., 2021). The presence of these compounds has increased interest in the valorisation of avocado seed as a potential source of functional ingredients and other value-added products rather than treating it solely as an agricultural waste material (Araújo et al., 2021; Nunes et al., 2026).

Recent studies have further highlighted avocado seed as a promising source of bioactive compounds for food, pharmaceutical, nutraceutical and other industrial applications (Rodríguez-Carpena et al., 2021).

Phytochemicals are naturally occurring secondary metabolites produced by plants and include phenolic compounds, flavonoids, tannins, alkaloids, terpenoids, saponins, steroids and glycosides. These compounds contribute to the biological properties of many plant materials and have been associated with antioxidant, antimicrobial, anti-inflammatory and other physiological activities (Dabas et al., 2013; Araújo et al., 2021). In avocado seed, phenolic compounds and flavonoids are particularly important because they constitute major components of its phytochemical profile and have been linked to its antioxidant and other biological properties (Rodríguez-Carpena et al., 2020 Nunes et al., 2026). The concentration and composition of these constituents, however, may vary depending on cultivar, geographical origin, maturity, processing conditions, extraction solvent and analytical procedure (Segovia et al., 2016; Araújo et al., 2021)

Conventional phytochemical screening provides useful preliminary information on the classes of secondary metabolites present in plant materials. However, such screening generally does not establish the identity and relative abundance of individual chemical constituents. More advanced analytical techniques are therefore required to characterize the chemical composition of plant extracts in greater detail. Gas Chromatography–Mass Spectrometry (GC–MS) is one of the analytical techniques widely employed for chemical profiling because it combines chromatographic separation with mass spectral characterization, allowing individual constituents in complex mixtures to be separated and tentatively identified (Hatzakis et al., 2019; Rodríguez-Carpena et al., 2020).

Previous GC–MS investigations have demonstrated that avocado seed contains a diverse range of chemical constituents, including fatty acids, terpenoid-related compounds and other lipophilic constituents (Hatzakis et al., 2019). For example, linoleic and linolenic acid derivatives have been detected among the lipophilic constituents of avocado seed, demonstrating the usefulness of chromatographic techniques in characterizing its chemical composition (Hatzakis et al., 2019). Differences in the compounds reported across studies may be related to differences in avocado variety, geographical origin, sample preparation, extraction conditions and analytical procedures (Hatzakis et al., 2019; Chen and Zhu, 2025).

The increasing interest in avocado seed valorisation has therefore created a need for detailed characterization of its phytochemical composition and individual chemical constituents. Although several studies have investigated avocado seed, differences in reported phytochemical profiles indicate the importance of characterizing samples from specific sources and under defined analytical conditions (Segovia et al., 2016; Hatzakis et al., 2019). Such characterization can provide a basis for understanding the chemical composition of the seed and identifying constituents of potential biological and industrial relevance.

Therefore, this study aimed to evaluate the phytochemical composition of avocado pear (*Persea americana*) seed and characterize functional group chemical constituents classification using Gas Chromatography–Mass Spectrometry (GC–MS).

## Materials and Methods

### Sample Collection

The study material consisted of mature seeds of *Persea americana* (avocado) obtained from ripe avocado fruits. The fruits were placed in sterile polyethylene bags, labelled, and transported to the laboratory and preserved for processing and subsequent phytochemical and GC–MS analyses. The plant material was properly identified and authenticated prior to analysis.

### Sample Preparation

The fresh avocado fruits were purchased from markets in Port Harcourt. The seeds were removed, washed, air-dried, and ground into fine powder. Soxhlet extraction was performed for both ethanolic and aqueous extracts as described previously.

Extracts were concentrated, dried, and stored at 4°C until use. The analysis was performed using a Gas Chromatograph coupled with a Mass Spectrometer following the method described by Adams (2007).

### Soxhlet Extraction of Avocado Seeds

The powdered *Persea americana* seed samples were subjected to Soxhlet extraction using ethanol as the extraction solvent. A known quantity of the powdered sample was placed in a Soxhlet extraction apparatus and continuously extracted with ethanol until adequate extraction was achieved.

The resulting extract was subsequently concentrated under reduced pressure using a rotary evaporator at approximately 40°C to remove the solvent and obtain the crude avocado seed extract. The concentrated extract was stored appropriately until required for quantitative phytochemical and GC–MS analyses (Hatzakis et al., 2019).

### Quantitative phytochemical Analysis

Qualitative phytochemical screening of the crude avocado seeds were carried out to determine the presence of selected secondary metabolites using standard procedures as described by Harborne (1998), Trease and Evans (2002), and Sofowora (2008). The extract was screened for alkaloids, flavonoids, tannins, saponins, terpenoids, and phenolic compounds;

#### Test for Alkaloids (Mayer's and Wagner's Tests)

Two millilitres of the extract was treated separately with a few drops of Mayer's reagent and Wagner's reagent. The formation of a cream or reddish-brown precipitate was indicated the presence of alkaloids (Trease and Evans, 2002).

#### Test for Flavonoids (Alkaline Reagent Test)

Two millilitres of the extract was mixed with a few drops of 10% sodium hydroxide solution. The appearance of an intense yellow coloration that becomes colourless upon the addition of dilute hydrochloric acid was indicated the presence of flavonoids (Harborne, 1998).

#### Test for Tannins (Ferric Chloride Test)

Two millilitres of the extract was treated with a few drops of 5% ferric chloride solution. The formation of a blue-black or greenish coloration was indicated the presence of tannins (Sofowora, 2008).

### Test for Saponins (Frothing Test)

Two millilitres of the extract was mixed with 5 mL of distilled water and shaken vigorously. Persistent frothing lasting for at least 15 minutes was indicated the presence of saponins (Trease and Evans, 2002)

### Test for Terpenoids (Salkowski Test)

Two millilitres of the extract was mixed with 2 mL of chloroform, followed by the careful addition of concentrated sulfuric acid along the side of the test tube to form a layer. A reddish-brown coloration at the interface was indicate the presence of terpenoids (Harborne, 1998).

### Test for Phenolic Compounds

Two millilitres of the extract was treated with a few drops of ferric chloride solution. The formation of a deep blue or black coloration was indicated the presence of phenolic compounds (Sofowora, 2008).

### Gas Chromatography–Mass Spectrometry (GC–MS) Analysis

The dried avocado seed extract was subjected to gas chromatography–mass spectrometry (GC–MS) analysis for the identification and characterization of its chemical constituents. The analysis was performed using a GC–MS system equipped with a capillary column and helium as the carrier gas. The extract was prepared for instrumental analysis by dissolving an appropriate quantity of the dried extract in a GC-compatible solvent and filtering the resulting solution through a 0.45µm PTFE membrane filter prior to injection.

A 1µL aliquot of the prepared sample was injected into the GC–MS system. Chromatographic separation was achieved using a DB-5MS or equivalent capillary column, with helium employed as the carrier gas at a flow rate of approximately 1.0 mL/min. The oven temperature was programmed from 50°C to 280°C using the specified temperature ramps to facilitate separation of the extract constituents. The mass spectrometer operated under electron-impact (EI) ionization at 70 eV, and mass spectra were acquired over an m/z range of 40–600 (Rodríguez-Carpena et al., 2020)

The compounds eluting from the chromatographic column were ionized and fragmented in the mass spectrometer to generate characteristic mass spectra. The resulting spectra were compared with reference spectra in the NIST mass spectral library for tentative identification of the detected constituents. Where applicable, compound identification was further supported by comparison of retention characteristics with available reference information (Adams (2007). The relative abundance of the identified compounds was estimated from their corresponding peak areas and expressed as percentage relative peak area (Segovia, et al., 2016).

### Experimental Design/Statistical Analysis

The quantitative phytochemical results were analysed using descriptive statistics and presented as mean values where replicate determinations were available. The results were expressed in their respective units and summarized in tables. The GC–MS data were analysed descriptively based on the retention time, identified compound and relative peak area (%) of each detected constituent. The relative peak area was used to indicate the relative abundance of individual compounds within the analysed extract.

### Results

The result of the quantitative phytochemical analysis of *Persea americana* seed is presented in Table 1. The phytochemical analysis revealed the presence of eight major phytochemical groups which were; alkaloids, flavonoids, saponins, tannins, terpenes, phenols, glycosides and steroids.

Phenolic compounds recorded the highest concentration (28.11 mg/100 g), followed by flavonoids (18.54 mg/100 g) and tannins (9.08 mg/100 g). Glycosides, steroids, alkaloids, saponins and terpenes occurred at comparatively lower levels, with values of 6.19, 3.47, 3.90, 2.15 and 1.67 mg/100 g, respectively.

The relatively high phenolic and flavonoid contents observed in this study are particularly relevant because avocado seed has been investigated as a potential source of naturally occurring bioactive compounds.

However, the concentration of individual phytochemicals can vary considerably depending on cultivar, geographical origin, maturity, extraction procedure and analytical method. Therefore, the values obtained in the present study represent the phytochemical profile of the particular avocado seed samples obtained from Etche Market and analytical conditions used.

**Table 1: Quantitative Phytochemical Composition of Avocado (*Persea americana*) Seeds from Etche Market**

| Phytochemical       | Avocado seed |
|---------------------|--------------|
| Alkaloid (mg/100g)  | 3.90         |
| Flavonoid (mg/100g) | 18.54        |
| Saponin (mg/100g)   | 2.15         |
| Tannin (mg/100g)    | 9.08         |
| Terpenes (mg/100g)  | 1.67         |
| Phenol (mg/100g)    | 28.11        |
| Glycoside (mg/100g) | 6.19         |
| Steroid (mg/100g)   | 3.47         |

### Gas Chromatography Mass-Spectrometry (GC–MS) Analysis of *Persea americana* Seed Extract

GC–MS analysis of the *Persea americana* seed extract revealed 26 compounds with varying relative peak areas (Table 2). The identified compounds comprised fatty acids and their derivatives, terpenoids/triterpenoids, sterols, phenolic compounds, alcohols and other organic constituents.

Linoleic acid recorded the highest relative peak area (13.78%), followed by oleic acid (10.92%), Hexadecanoic acid methyl ester (9.78%), geraniol (8.72%), hexanoic acid (6.70%), gallic acid (5.63%), and phytol (5.82%).

Other compounds detected at intermediate proportions included Decanoic acid (4.36%), campesterol (3.75%),  $\beta$ -sitosterol (3.15%), stigmasterol (2.58%), linalool (2.55%), catechin (2.73%), and furfural (2.84%).

The compounds with the lowest relative peak areas were  $\alpha$ -amyrin (0.63%), epicatechin (0.68%),  $\beta$ -amyrin (1.24%), dodecanoic acid (1.19%), and  $\beta$ -caryophyllene (1.13%).

Table 2: Gas Chromatography-Mass Spectrometry (GC-MS) Analysis of Avocado (*P. americana*) Seed Extract

| S/N                                        | Description                    | R.T<br>(min) | Molecular<br>Weight (g/mol) | Peak Area<br>(%) | Dev<br>(min) |
|--------------------------------------------|--------------------------------|--------------|-----------------------------|------------------|--------------|
| <b>A. Fatty acids and derivatives</b>      |                                |              |                             |                  |              |
| 1                                          | Linoleic acid                  | 25.784       | 280.45                      | 13.7             | 57           |
| 2                                          | Oleic acid                     | 14.841       | 282.12                      | 10.92            | 19           |
| 3                                          | Hexadecanoic acid methyl ester | 18.942       | 270.45                      | 9.78             | 48           |
| 4                                          | Hexanoic acid                  | 23.825       | 116.16                      | 6.70             | 22           |
| 5                                          | Decanoic acid                  | 25.463       | 172.26                      | 4.36             | 46           |
| 6                                          | Heptanoic acid                 | 25.927       | 130.18                      | 2.75             | 34           |
| 6                                          | Dodecanoic acid                | 23.576       | 200.32                      | 1.19             | 18           |
| <b>B. Terpenes and derivatives</b>         |                                |              |                             |                  |              |
| 7                                          | Geraniol                       | 21.769       | 154.25                      | 8.72             | 43           |
| 8                                          | Phytol                         | 10.219       | 296.53                      | 5.82             | 24           |
| 9                                          | Linalool                       | 20.853       | 154.25                      | 2.55             | 57           |
| 10                                         | Squalene                       | 17.957       | 410.73                      | 1.91             | 42           |
| 11                                         | $\beta$ -caryophyllene         | 22.218       | 204.35                      | 1.13             | 29           |
| 12                                         | $\beta$ -amyryn                | 18.619       | 426.72                      | 1.24             | 51           |
| 13                                         | $\alpha$ -amyryn               | 18.178       | 426.72                      | 0.63             | 39           |
| 14                                         | Lupeol                         | 20.425       | 426.72                      | 1.93             | 24           |
| <b>C. Phytosterols and derivatives</b>     |                                |              |                             |                  |              |
| 15                                         | Campesterol                    | 13.734       | 194.18                      | 3.75             | 33           |
| 16                                         | $\beta$ -sitosterol            | 10.852       | 414.71                      | 3.15             | 23           |
| 17                                         | Stigmasterol                   | 13.182       | 412.69                      | 2.58             | 19           |
| <b>D. Phenolic and flavonoid compounds</b> |                                |              |                             |                  |              |
| 18                                         | Gallic acid                    | 14.296       | 170.12                      | 5.63%            | 45           |
| 19                                         | Catechin                       | 15.738       | 290.27                      | 2.73%            | 22           |
| 20                                         | Quercetin                      | 12.492       | 302.24                      | 1.90%            | 15           |
| 21                                         | Epicatechin                    | 17.432       | 290.27                      | 0.68%            | 16           |
| <b>Other compounds</b>                     |                                |              |                             |                  |              |
| 22                                         | Furfural                       | 8.724        | 96.08                       | 2.84             | 26           |
| 23                                         | 5-Hydroxymethylfurfural        | 10.532       | 126.11                      | 1.46             | 31           |
| 24                                         | Acetic acid                    | 12.956       | 60.05                       | 1.63             | 20           |
| 25                                         | Carposide                      | 23.167       | 432.38                      | 1.58             | 35           |

## Discussion

The quantitative phytochemical analysis of *Persea americana* seed extract revealed the presence of alkaloids, flavonoids, saponins, tannins, terpenes, phenols, glycosides and steroids in varying concentrations. Among these phytochemicals, phenols (28.11 mg/100 g) were the most abundant, followed by flavonoids (18.54mg/100g) and tannins (9.08 mg/100g).

The abundance of these phytochemicals demonstrates that avocado seed is a rich source of bioactive compounds with potential antimicrobial properties.

This finding agrees with Rodríguez-Carpena *et al.* (2020), who reported that avocado seeds contain appreciable amounts of phenolic compounds responsible for their antioxidant and antimicrobial properties.

The high phenolic content observed in this study suggests that phenolic compounds may have played a major role in the antimicrobial activity exhibited by the avocado seed extract. Phenolic compounds are well known for their ability to inhibit microbial growth through disruption of cell membranes, enzyme inactivation and interference with microbial metabolic pathways.

This finding agrees with Rodríguez-Carpena et al. (2020), who reported that avocado seeds contain appreciable amounts of phenolic compounds responsible for their antioxidant and antimicrobial properties. Similarly, Araújo et al. (2021) observed that phenolic compounds were the predominant phytochemicals in avocado seed extracts and significantly contributed to their biological activities (Shahidi, and Ambigaipalan, 2015).

Flavonoids constituted the second most abundant phytochemical in the present study. Flavonoids possess broad-spectrum antimicrobial activities and have been reported to inhibit bacterial and fungal growth through membrane disruption, inhibition of nucleic acid synthesis and suppression of microbial enzymes. The present finding is consistent with the reports of Nwaoguikpe et al. (2014; Kumar et al. (2022), who identified flavonoids as major constituents of avocado seed extracts and attributed their antimicrobial activity largely to these compounds. The relatively high flavonoid concentration recorded in this study may therefore have enhanced the inhibitory activity of the extract against the isolated microorganisms.

Tannins were also detected in appreciable quantities. Tannins exert antimicrobial effects by forming complexes with microbial proteins, inactivating extracellular enzymes and disrupting cell wall integrity. This observation agrees with the findings of Daglia (2012) and Koul and Walia (2009), who reported that tannins inhibit the growth of several bacterial and fungal pathogens through protein precipitation and enzyme inhibition. Their presence in avocado seed extract may therefore have contributed synergistically to the observed antimicrobial activity.

Although alkaloids, saponins, glycosides, steroids and terpenes occurred in lower concentrations, their biological significance should not be underestimated. Alkaloids interfere with DNA replication and protein synthesis, while saponins increase membrane permeability, resulting in leakage of intracellular constituents.

Glycosides and steroids have also been reported to exhibit antimicrobial and antioxidant properties, particularly when acting synergistically with phenolic compounds.

Similar observations were reported by Cowan (1999) and Koul and Walia (2009), who emphasized that the antimicrobial efficacy of medicinal plants often results from the combined effects of several phytochemical constituents rather than a single active compound.

Overall, the quantitative phytochemical profile obtained in this study supports the growing evidence that avocado seed, often regarded as an agricultural by-product, possesses considerable pharmaceutical and agricultural value. The abundance of phenols, flavonoids and tannins provides a plausible explanation for the antimicrobial activity observed against microorganisms associated with diseased forest nursery seedlings (Shahidi, and Ambigaipalan, 2015)..

The GC–MS profile demonstrated that *P. americana* seed contains a chemically diverse mixture of fatty acids, terpenoids, sterols, phenolic compounds and other secondary metabolites. The predominance of fatty acids in the chromatographic profile is consistent with previous investigations showing that avocado seeds contain appreciable lipophilic constituents, including fatty acids and their derivatives Araújo, et al. (2021)

Linoleic acid had the highest relative peak area (13.78%), indicating that it was the most prominent compound detected under the conditions of the present GC–MS analysis. Linoleic acid is an unsaturated fatty acid commonly reported among the lipid constituents of avocado seed. Araújo, et al. (2021) for example, identified linoleic acid and related fatty-acid derivatives among the major compounds in avocado seed extracts.

The relatively high representation of linoleic acid in the present study therefore supports previous evidence that fatty-acid derivatives constitute an important component of the chemical profile of avocado seeds. This finding agrees with Desbois and Smith (2010), who reported that unsaturated fatty acids exhibit broad-spectrum antibacterial and antifungal activities by altering membrane permeability and inhibiting microbial metabolism.

Oleic acid (10.92%) was the second most abundant compound detected. Oleic acid is a monounsaturated fatty acid and has also been reported among the fatty-acid constituents of avocado seed preparations.

A recent investigation of avocado seed oil similarly identified oleic acid as one of its major GC–MS constituents, although the relative proportions differed from those observed in the present study. Such differences are expected because the chemical profile of plant materials may vary with cultivar, geographical origin, extraction procedure, sample preparation and analytical conditions.

The finding was the relatively high peak area of hexadecanoic acid methyl ester (9.78%), a methyl ester derivative of a fatty acid. Hexadecanoic acid methyl ester is the methyl ester form of palmitic acid and has been reported among the fatty-acid derivatives identified in plant extracts. Its relatively high representation in the present chromatographic profile indicates that fatty-acid derivatives constitute an important component of the avocado seed extract. Previous studies have reported hexadecanoic acid and its derivatives among the chemical constituents of *Persea americana* seed, although variations in their relative abundance may occur due to differences in cultivar, geographical origin, extraction procedures and analytical conditions. These observations are consistent with the reports of Cushnie and Lamb (2011), Sharifi-Rad *et al.* (2018) and Salehi (2019).

Geraniol (8.72%) was also detected at a relatively high proportion. Geraniol is an oxygenated monoterpene alcohol commonly found in plant essential oils and has been investigated for antimicrobial and other biological activities. Its presence, together with linalool (2.55%) and  $\beta$ -caryophyllene (1.13%), indicates the occurrence of volatile terpenoid constituents in the seed extract. The biological effects of avocado seed extracts have previously been associated with their complex mixture of phenolic and lipophilic constituents rather than with a single compound.

The analysis also detected several sterols, including campesterol (3.75%),  $\beta$ -sitosterol (3.15%) and stigmasterol (2.58%). These compounds are phytosterols and are important components of plant lipid fractions. Their detection further demonstrates the chemically diverse nature of the avocado seed extract. Squalene (1.91%), a triterpene hydrocarbon and precursor in sterol biosynthesis, was also detected.

The chromatogram contained several phenolic compounds, including gallic acid (5.63%), catechin (2.73%), quercetin (1.90%) and epicatechin (0.68%). These compounds are particularly relevant because phenolic acids and flavonoids are widely associated with antioxidant and antimicrobial properties. Previous work using chromatographic methods has confirmed the occurrence of gallic acid, quercetin and epicatechin among phenolic constituents of avocado seeds. The presence of these compounds in the present GC–MS profile is therefore consistent with previous chemical investigations of *P. americana* seed.

The detection of phytol (5.82%) is also noteworthy. Phytol is a diterpene alcohol and should not be classified as a phenolic compound. Its occurrence in the extract adds to the diversity of terpenoid constituents detected. This distinction is important when interpreting the GC–MS results alongside the quantitative phytochemical analysis, where phenol was measured as a phytochemical class (28.11 mg/100 g). The phenol result and phytol result represent different analytical categories and should not be equated.

The least represented compounds were  $\alpha$ -amyrin (0.63%) and epicatechin (0.68%). Although their relative peak areas were low, their detection remains relevant because biological activity is not necessarily determined solely by the abundance of an individual compound. Compounds occurring at lower proportions may contribute to biological effects through additive or synergistic interactions with other constituents. Previous studies of avocado seed extracts have similarly emphasized the contribution of multiple phenolic and other bioactive constituents to their biological properties.

The GC–MS findings demonstrate that *P. americana* seed contains a complex mixture of fatty acids, terpenoids, phytosterols and phenolic compounds. The predominance of linoleic acid, oleic acid, lupeol and geraniol, together with the presence of phytol, gallic acid, catechin, quercetin,  $\beta$ -sitosterol, campesterol and other constituents, provides a chemical basis for the biological potential observed for the seed extract. Previous studies Gallo, and Sarachine, (2009) have also demonstrated antimicrobial and antioxidant activities of avocado seed extracts, although the contribution of individual compounds requires further compound-specific investigation.

The GC–MS profile of *Persea americana* seed extract revealed a chemically diverse composition comprising fatty acids and fatty-acid derivatives, terpenes and terpenoids, triterpenoids, phytosterols, phenolic compounds and flavonoids. The predominance of linoleic acid (13.78%), oleic acid (10.92%) and hexadecanoic acid methyl ester (9.78%), together with the presence of compounds such as geraniol, phytol, gallic acid, lupeol,  $\beta$ -sitosterol, campesterol, catechin and quercetin, indicates the presence of constituents with recognised biological relevance. Previous GC–MS investigations of *P. americana* seeds have similarly identified fatty acids, fatty-acid derivatives and other lipophilic constituents, while demonstrating antioxidant and antimicrobial activities of avocado seed extracts (FAO. (2023).

Several of the chemical groups identified in the present study have been associated with antioxidant, antimicrobial and anti-inflammatory activities. Phenolic compounds and flavonoids are widely recognised for their antioxidant properties, while terpenoid constituents such as phytol and other related compounds have also been investigated for diverse biological activities (FAO, 2023; Evurani et al., 2025). The occurrence of these compounds alongside the abundant fatty acids suggests that the biological potential of the extract may arise from the combined contribution of several chemical constituents rather than from a single compound. The presence of diverse phytoconstituents may also provide a chemical basis for the antimicrobial activity observed in the present study. Indeed, previous research has demonstrated antimicrobial activity of avocado seed extracts against bacterial organisms, supporting the potential biological relevance of the complex chemical profile of the seed (FAO 2023). However, the relative peak areas obtained from GC–MS indicate the relative abundance of detected constituents and do not establish that any particular compound was responsible for the antimicrobial activity observed. Establishing such a relationship would require isolation and purification of individual compounds followed by compound-specific biological assays.

The identified constituents were grouped according to their chemical functional classes. The predominance of linoleic acid indicates that unsaturated fatty acids constitute an important part of the GC–MS profile of the extract.

Linoleic acid is a polyunsaturated omega-6 fatty acid, whereas oleic acid is a monounsaturated omega-9 fatty acid. Hexadecanoic acid methyl ester is the methyl ester derivative of palmitic acid.

These compounds represent structurally diverse terpenoid constituents. Phytol, for example, is a diterpene alcohol and not a phenolic compound. Lupeol and the amyrins are pentacyclic triterpenoids, while geraniol and linalool are oxygenated monoterpenes (Gallo, and Sarachine, 2009). These compounds are particularly important when interpreting the biological potential of the extract because phenolic acids and flavonoids are widely investigated for antioxidant and antimicrobial activities. These compounds contribute to the overall chemical diversity of the extract but should not automatically be interpreted as responsible for the biological activity observed (Gallo, and Sarachine, 2009).

## Conclusion

The GC–MS findings demonstrate that *P. americana* seed is a chemically rich plant material containing several classes of compounds with reported biological significance. The results therefore provide a chemical basis for further investigation of avocado seed as a potential source of bioactive compounds, while recognizing that the biological effects of the individual constituents and their possible synergistic interactions require further experimental validation.

## Recommendations

Based on the findings of the study, the following recommendations are made:

1. The major bioactive constituents, particularly the compounds occurring at relatively high peak areas, should be isolated and subjected to individual biological assays to establish their specific activities.
2. Research should be extended to the optimization and standardization of avocado seed extraction methods to improve the recovery of potentially valuable bioactive constituents.
3. The potential for valorisation of avocado seed as a source of bioactive compounds should be explored, considering that the seed is commonly discarded as an agricultural/food-processing waste material.

4. Further characterization of the identified compounds should be undertaken using advanced analytical techniques to confirm the identities of the GC–MS-detected constituents.
5. Further studies should investigate the antioxidant and antimicrobial activities of the avocado seed extract and determine the relationship between its phytochemical composition and observed biological effects.

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