"GC-MS PROFILING OF BIOACTIVE PHYTOCHEMICALS IN *PERSEA AMERICANA* FRUIT EXTRACTS AND THEIR PHARMACEUTICAL POTENTIAL"

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Abstract:

This study explores the bioactive phytochemicals present in fruit pulp extracts of *Persea americana* (avocado), using ethanol, petroleum ether, and chloroform as solvents. The pulp was macerated in each solvent, and the resulting extracts were analysed through Gas Chromatography–Mass Spectrometry (GC–MS) with a Clarus 680 system. Preliminary phytochemical screening confirmed the presence of key groups such as alkaloids, flavonoids, terpenoids, and phenolic compounds. Comparison of GC–MS spectra with the Vellore Institute of Technology (VIT) library revealed a diverse range of unique compounds, each varying with the solvent used. Many of these compounds are associated with significant biological and pharmacological functions. In addition, the extracts demonstrated antibacterial activity against selected pathogenic bacteria. The findings suggest that avocado pulp is a rich source of bioactive molecules and holds strong potential for future pharmaceutical and therapeutic applications.

Key Words: *Persea americana*, GC–MS, Ethanol extract, Petroleum ether extract, Chloroform extract, Bioactive compounds, Antibacterial activity.

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INTRODUCTION:

Persea americana Mill., commonly known as avocado, belongs to the Lauraceae family and is an evergreen tree native to Central and South America. Over time, it has been introduced to tropical and subtropical regions around the world, where it has gained recognition for its nutritional, economic, and medicinal importance. The fruit is a single-seeded, pear-shaped berry, covered by a smooth or rough outer skin, and filled with a creamy pulp. This flesh is particularly valued for its richness in monounsaturated fatty acids, phytosterols, vitamins, minerals, and antioxidants (Afzal *et al.,* 2022). Because of its buttery texture and nutty flavour, avocado is popularly referred to as 'butter fruit'.

In India, avocado cultivation has been successfully established in the Eastern Himalayas, where humid subtropical conditions and moderate summer rainfall provide a favourable environment for growth. Typically, cultivation occurs between September and March, aligning with optimal agro-climatic conditions (Subba *et al.,* 2023). Globally, research on avocado continues to expand, covering various aspects such as climate adaptability, pest and disease management, oil extraction methods, nutrition, and sustainable farming practices. These ongoing efforts emphasize its growing significance in both health-related applications and commercial industries (Afzal *et al.,* 2022).

TAXONOMICAL ORDER:

Kingdom: Plantae

Phylum: Angiosperm

Class: Eudicots

Order: Laurale

Family: Lauraceae

Genus: *Persea*

Species: *americana*

Fig 1: Avocado fruit

NUTRIENT VALUE:

A 100 g serving of avocado fruit provides approximately 160 kcal of energy, along with 8.5 g carbohydrates, 2 g protein, 14.7 g fats, and 6.7 g dietary Fiber. It also contains calcium (12 mg), iron (0.6 mg), magnesium (29 mg), phosphorus (5 mg), potassium (485 mg), and zinc (0.6 mg). Avocado is cholesterol-free and holds about 73.2 g of water per 100 g of pulp, with natural sugars accounting for only 0.7 g.

The pulp is enriched with essential vitamins, including vitamin C (10 mg), pantothenic acid (1.5 mg), pyridoxine (0.3 mg), folic acid (81 µg), vitamin K (21 µg), vitamin E (21 mg), and vitamin A (146 IU). This diverse nutritional profile makes avocado a functional food with potential health-promoting properties

MEDICINAL USES:

Traditionally, avocados have been used in folk medicine across Central and South America, their native regions. Over time, their medicinal applications have spread globally and are now incorporated into herbal, naturopathic, and functional medicine practices.

Modern research has confirmed a wide range of health benefits, supported by both in vitro and in vivo studies:

- **Heart Health** – Avocado consumption improves lipid profiles and cardiovascular function (Dreher & Davenport, 2013; Dreher, 2018).
- **Anti-inflammatory Properties** – Bioactive compounds reduce inflammation in vitro and in vivo (Lu *et al.,* 2005).
- **Digestive Health** – High fibre content supports gut health and digestion (Dreher & Davenport, 2013).
- **Skin Health** – Avocado oil and extracts show protective effects in dermatological applications (Dreher, 2018).
- **Weight Management**– Avocados promote satiety and reduce abdominal fat distribution (Dreher & Davenport, 2013).
- **Blood Pressure Regulation** – Rich potassium content contributes to lowering blood pressure (Navarro *et al.,* 2002).
- **Anti-Cancer Potential** – Avocado extract inhibits growth of prostate cancer cells and other malignancies (Lu *et al.,* 2005).
- **Eye and Oral Health** – Lutein and phytochemicals improve eye health and reduce oxidative stress in oral tissues (Dreher, 2018).
- **Bone Health** – Nutrient profile supports calcium absorption and bone strength (Schaffer *et al.,* 2013).
- **Nutrient Absorption** – Monounsaturated fats enhance absorption of carotenoids and other nutrients (Dreher, 2018).

MATERIALS AND METHODS:

The fruit is sourced from Bangalore city. The pericarp is first removed, and the pulp of the fruit is used for further investigation. The extraction process is carried out using solvents such as ethanol, petroleum ether, and chloroform. The resulting extract are filtered through Whatman filter paper 40 and then concentrated using a rotatory evaporator. The extracts are analysed for the presence of alkaloids, flavonoids, phenols, and terpenoids. Additionally, GC-MS analysis and antibacterial activity tests are performed on the extracts(Yaswanth *et al.*,2021).

Phytochemical Screening:

Test for Alkaloids: To 5 ml of avocado extract, 2 ml of concentrated HCl is added. To this acidic solution, 1 ml of Dragendorff's reagent is added. The formation of an orange-brown precipitate confirms the presence of alkaloids in avocado extracts (Li *et al.,* 2023).

Test for Flavonoids : To 1 ml of avocado extract, a few drops of dilute sodium hydroxide solution are added. The development of a yellow colour indicates the presence of flavonoids, which are abundant in avocado mesocarp and seed (Cao *et al.,* 2014).

Test for Terpenoids : To 0.5 ml of avocado extract, 2 ml of chloroform is added, followed by concentrated sulfuric acid. The formation of a reddish-violet colour confirms the presence of terpenoids, which are known bioactive compounds in avocado (Rahdar *et al.,* 2020).

Test for Phenols : To 1 ml of avocado extract, 2 ml of distilled water is added, followed by a few drops of ferric chloride solution. The appearance of blue or green colour indicates the presence of phenolic compounds, consistent with avocado's strong antioxidant profile (Wen *et al.,* 2013).

GC-MS Analysis: The Clarus 680 GC was used in the analysis employed a fused silica column, packed with Elite-5MS (5% biphenyl 95% dimethylpolysiloxane, 30m × 0.25mm ID × 250µm df) and the components were separated using Helium as carrier gas at a constant flow of 1ml/min.

ACQUISITION PARAMETERS:

Oven: initial temp 60°C for 2min, ramp10°C/min to 300°C, hold 6 min,

Total Run Time: 32.00 mint **Inauto:** 260°C

Volume: 1 µL, **Flow Rate:** 1 mL/mint

Carrier Gas: He, **Column:** Elite- 5MS(30.0m, 0.25mmID, 250µm df)

MASS CONDITION(EI):

Solvent Delay: 2.00min, **Transfer Temp:** 240°C,

Source Temp: 240°C, **Scan:** 50 to 600Da

Identification of chemical constituents: The spectrums of the components were compared with the database of the spectrum of known components stored in the GC-MS NIST (2008) library.

Antibacterial Screening:

Three types of bacteria were chosen for testing because they are important for medicine and health: *Escherichia coli, Salmonella typhi,* and *Klebsiella pneumoniae*.

The bacteria cultures were grown in nutrient broth at 37°C and on nutrient agar. The agar slants were prepared and stored at 4°C. These cultures were sourced from Bharathi Women's College, Chennai. Using the well diffusion method, the antibacterial activity of ethanol, petroleum ether and chloroform extracts were assessed.

Using sterile swabs, 40µl of a bacterial solution(1.65×10^6 CFU/ml) was spread on agar plates for inoculation. A sterile tool was used to make 6mm wells in the agar. Then, different amounts of solvent extracts dissolved in Dimethyl Sulphoxide(DMSO) were added to each well(50µl, 100µl, 150µl, 200µl, and 250µl). Ampicillin was used as a positive control, while DMSO was used as a negative control.

Statistical Analysis: Each experiment was conducted in three duplicates. The findings are presented as mean ± standard errors and one-way analysis of variants (ANOVA) was used to compare the samples' antibacterial activity with standard antibiotics.

RESULTS AND DISCUSSION:

Analysis of Phytochemicals: The presence of Alkaloids, Flavonoids, Phenols and Terpenoids in chloroform, ethanol and petroleum ether are exposed by phytochemicals screening which were tabulated in **Table 1.**

Table 1: Phytochemical analysis of *Persea americana*.

S.no	Phytochemicals	Chloroform Extract	Ethanol extract	Petroleum Ether Extract
1	Alkaloids	Present	Present	Present
2	Flavonoids	Present	Present	Absent
3	Phenols	Present	Present	Absent
4	Terpenoids	Absent	Absent	Absent

Presence of Bioactive Compounds in the Extracts: The compounds were identified and characterized based on their elution order in the Elite-5MS column. The bioactive compounds found in the Ethanol extract are listed in **Table 2.**

The table below provides information about the retention time, compound name, percentage area, and molecular formula. **Fig 2** shows the chromatogram of the ethanol extract.

The top two compounds based on the richness in the ethanol extract are E-2-Octenyl tiglate and 3- Dodecen-1-ol. The physicochemical properties of one of the compounds which are listed in **Table 3.**

Table 2: Bioactive compounds of Ethanol Extract

Retention Time	Name of the compound	Molecular Formula	Area %
17.465	13-Tetradecyonic Acid, Methyl Ester	$C_{15}H_{26}O_2$	2.706
18.041	E-2-OCTENYL TIGLATE	$C_{13}H_{22}O_2$	48.840
18.796	9,12-Octadecadienoic Acid, Methyl Ester (E, E)-	$C_{19}H_{34}O_2$	1.224
18.986	Cyclohexylmethanol, Trifluoroacetate (Ester)	$C_9H_{13}O_2F_3$	2.808
19.536	1,15-Hexadecadiene	$C_{16}H_{30}$	1.810
19.641	3-Dodecen-1-ol	$C_{12}H_{24}O$	34.816
20.362	8-Heptadecene, 1-chloro-	$C_{17}H_{33}Cl$	1.243

Table 3: Physicochemical properties of E-2-Octenyl Tiglate

Formula	$C_{13}H_{22}O_2$
Molecular Weight	214.31 g/mol
Num. heavy atoms	15
Fraction Csp3	0.92
Num. rotatable bonds	5
Num H-bond acceptors	2
Num H-bond donors	0
Molar Refractivity	66.96
TPSA	28.8 A^2
GI absorption	Low
BBB permeant	Yes
Lipinski	Yes

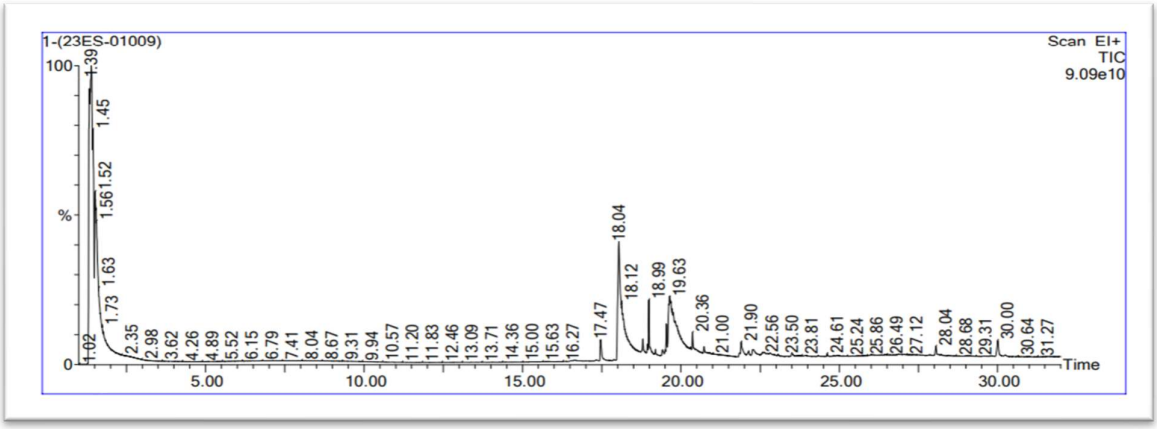


Fig 2: Ethanol Extract Gas Chromatography

Presence of bioactive compounds in the Extracts: The compounds were identified and characterized based on their elution order in the Elite-5MS column. The bioactive compounds present in the chloroform extract are listed in **Table 4.**

The table below provide the retention time, compound name, percentage area and molecular formula. Figure 3 displays the chromatogram of the chloroform extract.

The top two compounds based on the richness in the ethanol extract are (R)-(-)-4-Methylhexanoic Acid and Cis-2-Methyl-7-Octadecene . The physicochemical properties of one of the compounds which are listed in **Table 5.**

Table 4: Bioactive compounds of Chloroform extract

Retention time	Name of the compound	Molecular Formula	Area %
1.569	Thiazolidine, 2-Ethyl-	$C_5H_{11}NS$	8.606
1.609	Ethanol, 2-[(1-Methylethyl)Amino-]	$C_5H_{13}ON$	7.491
17.415	Hexanoic Acid, 2-Methyl-	$C_7H_{14}O_2$	2.126
18.010	(R)-(-)-4-METHYLHEXANOIC ACID	$C_7H_{14}O_2$	29.909
18.781	Cyclohexane, 1-(1,5-Dimethylhexyl)-4-(4-Methylpentyl)-	$C_{20}H_{40}$	7.671
18.966	Eicosanebioic Acid, Dimethyl ester	$C_{22}H_{42}O_4$	2.077
19.351	7-Decen-2-one	$C_{10}H_{18}O$	4.967
19.611	Cis-2-Methyl-7-Octadecene	$C_{19}H_{38}$	13.794
19.726	1-Hexyl-2-Nitrocyclohexane	$C_{12}H_{23}O_2N$	12.322
21.792	1-Hexyl-2-Nitrocyclohexane	$C_{12}H_{23}O_2N$	1.197
21.857	1-Hexyl-2-Nitrocyclohexane	$C_{12}H_{23}O_2N$	2.240
22.167	1-Hexyl-2-Nitrocyclohexane	$C_{12}H_{23}O_2N$	3.481
22.667	1-Hexyl-2-Nitrocyclohexane	$C_{12}H_{23}O_2N$	1.929
30.020	1-Hexyl-2-Nitrocyclohexane	$C_{12}H_{23}O_2N$	2.191

Table-5 Physicochemical properties of (R)-(-)-4-METHYLHEXANOIC ACID

Formula	$C_7H_{14}O_2$
Molecular Weight	130.19 g/mol
Num. heavy atoms	9
Fraction Csp3	1.0
Num. rotatable bonds	3
Num H-bond acceptors	2
Num H-bond donors	1
Molar Refractivity	40.75
TPSA	$40.57A^2$
GI absorption	Medium
BBB permeant	No
Lipinski	Yes

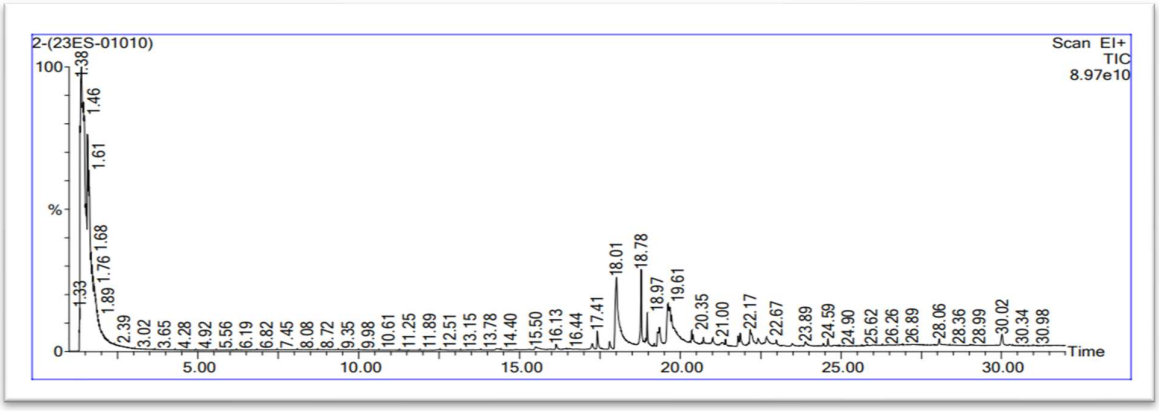


Fig 3: Chloroform Extract Gas Chromatography

Presence of Bioactive Compounds in the Extract: The Compounds in the Petroleum Ether extract were identified and characterized based on their elution order on the Elite-5MS Column, and the bioactive compounds are listed in **Table 6.**

The table below provide the retention time, compound name, percentage area and molecular formula. Figure 4 displays the chromatogram of the petroleum ether extract.The top two compounds based on the richness in the ethanol extract are Divinyl Sulphone and Oleic Acid. The physicochemical properties of one of the compounds which are listed in **Table 7.**

Table 6: Bioactive Compounds of Petroleum Ether Extract

Retention Time	Name of the compound	Molecular Formula	Area %
1.739	1,6;3,4-Dianhydro-2-Deoxy-.Beta.-D-Lyxo-Hexopyranose	$C_6H_8O_3$	1.098
17.540	13-Tetradecynoic Acid, Methyl Ester	$C_{15}H_{26}O_2$	1.216
18.055	DIVINYL SULPHONE	$C_4H_6O_2S$	30.156
18.831	3,7-Octadien-2-one,(E)-	$C_8H_{12}O$	1.432
19.016	Monomethyl pimelate	$C_8H_{14}O_4$	1.053
19.436	5-Butyl-1,3-Oxathiolan-2-one	$C_7H_{12}O_2S$	1.312
19.671	Oleic Acid	$C_{18}H_{34}O_2$	24.255
20.391	Hexadecanoic Acid, (3-Bromoprop-2-ynyl)Ester	$C_{19}H_{33}O_2Br$	1.178
21.917	9-Eicosyne	$C_{20}H_{38}$	1.523
23.547	Bis-(3,5,5-Trimethylhexyl)Phthalate	$C_{26}H_{42}O_4$	2.090
24.778	1,2-Benzenedicarboxylic Acid, Disodecyl Ester	$C_{28}H_{46}O_4$	0.955
30.040	1-Hexyl-2-Nitrocyclohexane	$C_{12}H_{23}O_2N$	2.371

Table 7: physicochemical properties of Divinyl Sulphone

Formula	$C_4H_6O_2S$
Molecular Weight	134.16 g/mol
Num. heavy atoms	7
Fraction Csp3	0.25
Num. rotatable bonds	2
Num H-bond acceptors	2
Num H-bond donors	1
Molar Refractivity	34.3
TPSA	$50.0A^2$
GI absorption	Low
BBB permeant	No
Lipinski	Yes

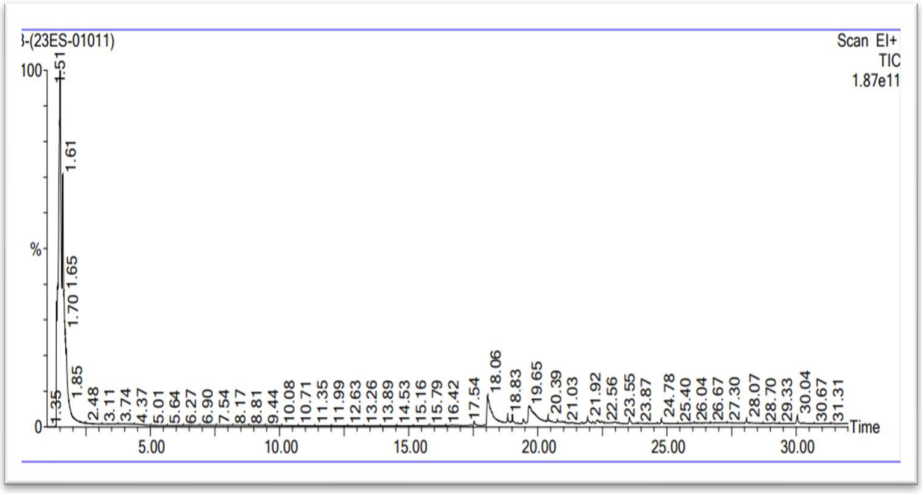
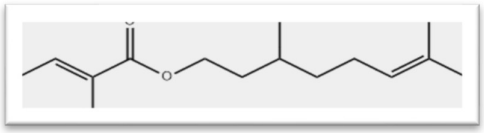
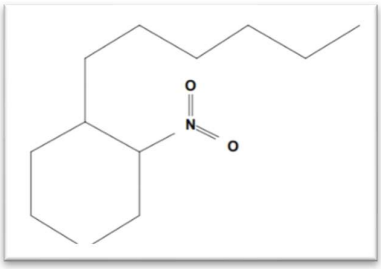


Fig 4: Petroleum Ether Extract Gas Chromatography

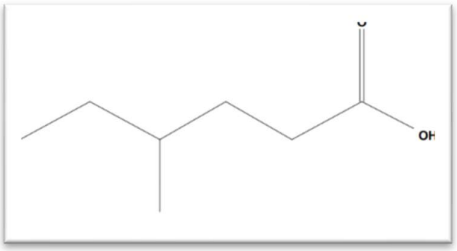
E-2-OCTENYL TIGLATE



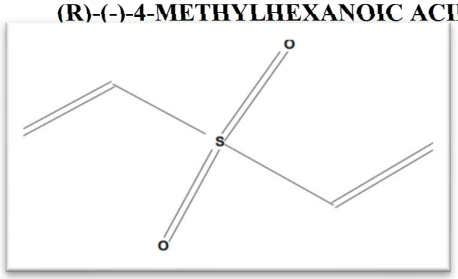
8-HEPTADECENE, 1-CHLORO



(R)-(-)-4-METHYLHEXANOIC ACID



1-HEXYL-2-NITROCYCLOHEXANE



DIVINYL SULPHONE

MONOMETHYL PIMELATE

Fig 5: Structures of Some of the Compounds Scrutinized by GC-MS

Phytochemical screening confirmed the presence of flavonoids and phenols in ethanol and chloroform extracts, whereas petroleum ether extract lacked these compounds. Terpenoids were not detected in any extract.

The GC–MS analysis of plant extracts identified unique bioactive compounds with promising drug-like potential. E-2-Octenyl Tiglate exhibits antioxidant and anti-inflammatory properties, consistent with findings from(Shoily *et al.,*2025), who reported strong bioactivity and Lipinski compliance in chloroform fractions of Heliotropism indicum . Similarly, (R)-(−)-4-Methylhexanoic Acid shows metabolic benefits comparable to 6-hydroxyhexanoic acid (6-HHA),

which reduced fat accumulation and improved insulin sensitivity in high-fat diet models (Sebag *et al.,* 2024;). Further, dietary medium-chain fatty acids were shown to lower hepatic triglycerides through the axis (Cao *et al.,* 2024;). Collectively, these findings support the therapeutic potential of the identified compounds

Antibacterial Activity: The antibacterial activity of the chloroform, ethanol, and petroleum ether extracts was evaluated using the well diffusion method, with Ampicillin as the positive control and DMSO as the negative control. The results, in the form of zone of inhibition measurements, are documented in **Tables 8, 9, and 10.**

Table 8: Antibacterial activity of Ethanol Extract

Pathogen	Concentration and Zone of inhibition					Ampicillin 5mg/ml
	50µL	100µL	150µL	200µL	250µL	
Escherichia Coli	5.4±0.4	4.7±0.2	6.4±0.4	7.5±0.5	14.5±0.2	23
Salmonella typhi	5.9±0.5	6.7±0.4	7.4±0.2	9.6±0.2	15.5±0.5	23
Klebsiella pneumonia	5.2±0.5	6.1±0.5	7.4±0.2	8.7±0.2	16.4±0.5	23

Data given are mean of three values ± Standard error.

Table 9: Antibacterial activity of Chloroform Extract

Pathogen	Concentration and Zone of inhibition					Ampicillin 5mg/ml
	50µL	100µL	150µL	200µL	250µL	
Escherichia Coli	5.6±0.4	4.4±0.5	5.7±0.8	9.2±0.2	19.4±0.4	24
Salmonella typhi	3.5±0.2	4.4±0.4	5.4±0.2	6.2±0.2	8.5±0.5	24
Klebsiella pneumonia	4.7±0.5	5.1±0.5	6.2±0.2	8.9±0.2	15.6±0.5	24

Data given are mean of three values ± Standard error.

Table 10: Antibacterial Activity of Petroleum Ether Extract

Pathogen	Concentration and Zone of inhibition					Ampicillin 5mg/ml
	50µL	100µL	150µL	200µL	250µL	
Escherichia Coli	4.7±0.4	5.5±0.5	7.4±0.2	9.1±0.5	12.4±0.2	24
Salmonella typhi	5.1±0.2	6.2±0.4	7.4±0.2	9.5±0.2	12.4±0.5	24
Klebsiella pneumonia	5.7±0.5	6.5±0.5	8.4±0.2	9.5±0.2	12.4±0.5	24

Data given are mean of three values ± Standard error.

CONCLUSION :

Avocado (*Persea americana*) is a nutritionally rich fruit with significant medicinal value. The GC–MS profiling of its pulp extracts revealed several bioactive compounds with potential pharmacological applications, particularly in antioxidant, anti-inflammatory, and antimicrobial therapies. These findings reinforce the role of avocado as a promising candidate in both nutritional and pharmaceutical fields**.**

CONFLICTS OF INTEREST : The authors declare no conflicts of interest.

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