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Microchemical analysis in fixed and volatile oil of black pepper (*Piper nigrum* L)

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ABSTRACT

The experiment on microchemical analysis of seed powder of black pepper was performed at FTL, JAU Junagadh. and the result shown the presence of 44 peaks in volatile oil extracted with rotary evaporator in powdered materials of the Piper nigrum L and 17 peaks of fatty acids in fixed oils in Soxhlet extracted oil. Most of them were found to be as intense pungent and fragrance compounds. The class of compounds in this oil were includes Aliphatic halide, Ketone, Alkane, Monoterpenoid alcohol, Alcohol (3), Bicyclic compound, Aromatic aldehyde, Aromatic ether, Aromatic hydrocarbon, Cycloalkene (4), Bicyclic hydrocarbon, Bicyclic alkene, Diene. The key Bio-marker compounds were found to be as the Caryophyllene at 21.889 minutes with peak number 29. The highest area, covering 23.21 %, was observed at 9.693 minutes, in addition to peak number 7, which showed the highest height with an area % of 20.84 at 11.51 minutes RT, and had 44 peaks in it adducts. These findings also shown the complete pictures of fatty acids too and the highest area% 32.91with 9,12-Octadecadienoic acid (PUFA). The quality oil indices were found to be in the range of SFA (36.65%), MUFA (25.81%), PUFA (37.54) %, MUFA/PUFA ratio (0.69), O/L ratio (6.24) and UnsFA/SFA (38.24). The literature cited here indicated compounds had antimicrobial, antioxidant, anti-inflammatory and insect-related activities as well as anticancer compounds too in black pepper

Keywords: Cardamom, GC MS, fixed oil and volatile oil

INTRODUCTION

Black pepper (*Piper nigrum* L.) is a tropical vine that can grow to a height of 4-9 m, but with the support of other trees. The plant has a shallow root system. Mostly Black pepper fruit is ball shaped, 8 mm in diameter, brown with a mesh-wrinkled surface. It has a spicy taste, a specific smell. These being used in India as spicy supplements. Generally, the major portion of this is used in food preparation as food additives as pungency. Round black rough balls are wholly or sometimes crushed powdered are also used because of its flavour and health effects. The aroma of pepper comes from the essential oil present in the fruit, while the presence of the alkaloids, mainly piperine, is important for its spiciness. There are numerous scientists had worked in black paper one of the vast data created by Van ruth *et al.* (2019) studied on 90 black pepper and 40 white peppers analysed through proton transfer reaction-mass spectrometry, the black pepper samples had notably higher intensities for 41% (52 out of 128) of the masses compared with white pepper. Many studies had proved the anticancer activity especially of piperine, against different cancer cell lines, The ethanolic extract of the black pepper fruit was found to have an anticancer effect on the three colorectal cancer cell lines: HT-29, HCT-116, and HCT-15 (Stojanovic-Radic *et al*. 2019 and Prashant *et al*. 2017). Most of the volatile organic compounds were found at higher concentrations in black pepper than in white pepper, except for five low-intensity compounds. Similarly, Lee *et al.* (2020) reported shows that pericarp has a fruitful flavour than the other parts, as determined by GC/MS analysis. Even though the regional and agricultural practices also differ in plants. Sometimes also extraction procedure affects for detection. (Tran) Therefore, our aim was to determines the important and key bioactive compounds in marketed black pepper of Indian culinary purpose. The food like biryani, Kava, juice extracts for relief or pre-digester and many pungent foods prepared from these ingredients so, it is prime necessity to level of compounds present in this Piper nigrum L. also to identify the other healthier aspects digging from the black seeds and their proportion untargeted compounds detection was also one of the aims behind these experiments. The compounds

detected through this GC MS have any pharmaceutical values or note. Such hypothesis was kept in mind during designing of this experiment

MATERIAL AND METHODS

Extraction: Methanolic extracts of powdered form of black pepper seeds were used. The Fixed oil was extracted through hexane in rotary evaporator also used for the analysis. The data showed 5.4 % oils in dry weight basis.

Instruments programme and conditions: The GC–MS system consisted of a Shimadzu QP2010S mass spectrometer with an autosampler (Shimadzu, Kyoto, Japan). The column temperature was programmed as follows: 60 °C, hold for 0 min, ramp at 150 °C/1min to 240 °C, hold for 5 min. Column Oven Temp.: 60.0 °C, Injection Temp.: 250.00 °C, Injection Mode: Split, Flow Control Mode: Pressure, Pressure: 57.4 kPa, Total Flow: 54.0 mL/min, Column Flow: 1.00 mL/min, Linear Velocity: 36.5 cm/sec. Purge Flow: 3.0 mL/min, Split Ratio: 50.0; High Pressure Injection, Carrier Gas Saver, Splitter Hold :OFF for GCMS-QP2010 Plus:, Insources: 230.00 °C, Interface Temp.: 240.00 °C, Solvent Cut Time: 2.00 min, Detector Gain Mode: Relative, Detector Gain: +0.00 kV, Threshold: 1000 for MS table; Start Time: 2.30min End Time: 31.50 min, ACQ Mode: Scan, Event Time: 0.50sec, Scan Speed: 2000, Start m/z: 50.00, End m/z: 1000.00. The compounds were match with time and mass spectra from existing literature and the mass spectral libraries from (NIST) National Institute of Standards Technology.

Detection of compound: The protocol used for GC MS analysis were according to Rathod *et al*. (2025). according to (NIST) National Institute of Standards Technology. Library compounds was detected and remove the methyl ester name in fatty acids and synonymous name was used for search the biological activities and their application

RESULTS AND DISCUSSION

For the GC–MS analysis of volatile and fixed oils of Black pepper through micro chemical analysis was done through basic principle of untargeted compounds identified based on the comparison of their retention time and mass spectra from existing literature and the mass spectral libraries from (NIST) National Institute of Standards Technology. In this experiment 44 volatile flavour compounds and their peak areas were determined (Table 1) and chromatogram of the peaks of compounds detected in the black pepper are presented in figure 1.

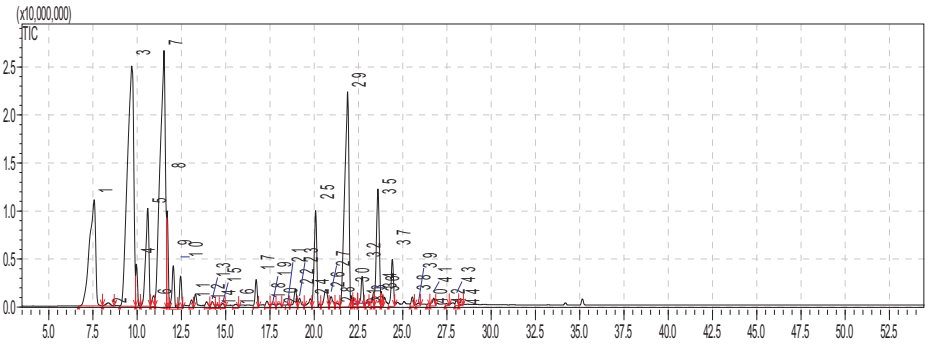


Figure chromatogram of the peaks of compounds detected in the black pepper

Peak No	RT (min)	Area (%)	Compound Identified
1	7.572	9.99	(1R)-2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene
2	8.363	0.23	Camphene
3	9.693	23.21	Bicyclo[3.1.0]hexane, 4-methylene-1-(1-methylethyl)-
4	9.953	1.06	β.-Myrcene
5	10.591	4.64	.α.-Phellandrene
6	10.921	0.45	1,3-Cyclohexadiene, 1-methyl-4-(1-methylethyl)-
7	11.51	20.84	Cyclohexene, 1-methyl-4-(1-methylethenyl)-, (S)-
8	11.695	1.93	Bicyclo[3.1.0]hexane, 4-methylene-1-(1-methylethyl)-

Peak No	RT (min)	Area (%)	Compound Identified
9	12.037	1.68	Benzene, 1-methyl-4-(1-methylethyl)-
10	12.453	1.08	1,4-Cyclohexadiene, 1-methyl-4-(1-methylethyl)-
11	13.072	0.37	Terpineol, Z-.β.-
12	13.918	0.35	1,6-Octadien-3-ol, 3,7-dimethyl-
13	14.236	0.35	Terpineol, Z-.β.-
14	14.527	0.18	Benzene, 1-methyl-4-(1-methylethenyl)-
15	14.831	0.25	2-Cyclohexen-1-ol, 1-methyl-4-(1-methylethyl)-, trans-
16	15.572	0.26	2-Cyclohexen-1-ol, 1-methyl-4-(1-methylethyl)-, cis-
17	16.716	0.96	3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)-
18	17.329	0.32	(+)-.α.-Terpineol (p-menth-1-en-8-ol)
19	17.642	0.25	2-Cyclohexen-1-ol,3-methyl-6-(1-methylethyl)-, trans-
20	18.021	0.25	Bicyclo [3.1.0]hexan-3-ol,4-methylene-1-(1-methylethyl)-, (1.α.,3.α.,5.α.)-
21	18.487	0.15	Cyclohexanone, 2-methyl-5-(1-methylethenyl)-
22	18.941	0.82	Cyclohexene,4-ethenyl-4-methyl-3-(1-methylethenyl)-1-(1-methylethyl)-, (3R-trans)-
23	19.168	0.42	α.-Cubebene
24	19.78	0.48	Benzaldehyde, 4-(1-methylethyl)-
25	20.077	3.88	Copaene
26	20.663	1.01	1H-Cycloprop[e]azulene, decahydro-1,1,7-trimethyl-4-methylene-, [1aR-(1a.α.,4a.β.,7.α.,7a.β.,7b.α.)]-
27	20.957	0.51	Benzene, 1-methoxy-4-(1-propenyl)-
28	21.241	0.21	Tetradecane, 1-chloro-
29	21.889	13.58	Caryophyllene
30	22.082	0.09	1,6,10-Dodecatriene, 7,11-dimethyl-3-methylene-, (E)-
31	22.224	0.02	Pentadecane, 4-methyl-
32	22.705	1.09	α.-Caryophyllene
33	22.995	0.18	GUAIENE <TRANS-B->
34	23.365	0.42	1,3-Cyclohexadiene, 5-(1,5-dimethyl-4-hexenyl)-2-methyl-, [S-(R@,S@)]-
35	23.599	5.23	Cyclohexene, 1-methyl-4-(5-methyl-1-methylene-4-hexenyl)-, (S)-
36	23.78	0.37	Naphthalene, 1,2,4a,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-
37	24.415	1.68	Naphthalene, 1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-, (1S-cis)-
38	25.54	0.31	Gamma.-Elemene
39	25.887	0.13	Dodecane, 2-methyl
40	26.472	0.08	Heptadecane, 3-methyl
41	26.757	0.33	Caryophyllene oxide
42	27.471	0.09	12-Oxabicyclo[9.1.0] dodeca-3,7-diene, 1,5,5,8-tetramethyl-, [1R-(1R@,3E, 7E,11R@)]-
43	28.045	0.2	1-Naphthalenol, 1,2,3,4,4a,7,8,8a-octahydro-1,6-dimethyl-4-(1-methylethyl)-, [1R-(1.α.,4.β.,4a.β.,8a.β.)]-
44	28.3	0.07	7-Hexadecenal, (Z)-

The major components were including with higher area % covered by four compounds viz., detected as (1R)-2,6,6-Trimethylbicyclo [3.1.1] hept-2-ene (9.99%), Bicyclol [3.1.0] hexane, 4-methylene-1-(1-methylethyl) -(23.21%), Cyclohexene, 1-methyl-4-(1-methylethenyl)-, (S)-(20.84%), Caryophyllene (13.58%) are terpenes. The (1R)-2,6,6-Trimethylbicyclo [3.1.1] hept-2-ene (L-.α.-Pinene); Cyclohexene, 1-methyl-4-(1-methylethenyl)-, (S)-(+)-Limonene; and Caryophyllene as the bicyclic sesquiterpene and Cyclohexene, 1-methyl-4-(5-methyl-1-methylene-4-hexenyl)-, (S)- is also a class of organic compounds known as sesquiterpenoids. Similar observation also made by Liang *et al*. (2021) These results are in agreements with Lee *et al* (2020), they detected lesser compounds but most of them were as founds in these experiments. And the proportion of they detected were α pinene (9.2%), 2 β pinene (14.3%), δ 3 carene (21.5%) and dl limonene (18.8%), and the primary sesquiterpenes were α copaene (5.1%) and caryophyllene (17.2%). The higher percentages of flavour compounds found in the pericarp would impart a more potent odour. Here Caryophyllene was lesser found from as Lee *et al* (2020. According to class of compounds, The *Piper nigrum C*ontained these chemical classes of compounds viz., Aliphatic halide, Ketone, Alkane, Monoterpenoid alcohol, Alcohol (3), Bicyclic compound, Aromatic aldehyde, Aromatic ether, Aromatic hydrocarbon, Cycloalkene (4),Bicyclic hydrocarbon, Bicyclic alkene, Diene, Out of 44 compounds nine were terpen and their derivatives including Terpene oxide, Terpene, Monoterpene (3),Sesquiterpene (4-5), Monoterpenoid alcohol, 2-Polycyclic aromatic hydrocarbon (PAHs), Polycyclic hydrocarbon. too. Similar findings with the compounds with biological activities of Black pepper. Similar observation also made by metabolic extracted from Piper also used GC–MS and LC-MS methods (Liang *et al.,* 2021).

Table.2 Volatile compounds formula and class in the black pepper through GCMS

Peak No.	Compound name	RT (min)	Area (%)	Chemical formula	Chemical Class
1.	Trimethylbicyclo[3.1.1]hept-2-ene	7.572	9.99	$C_{10}H_{16}$	Bicyclic alkene
2.	Camphene	8.363	0.23	$C_{10}H_{16}$	Monoterpene
3.	Bicyclo[3.1.0]hexane	9.693	23.21	$C_{10}H_{16}$	Bicyclic hydrocarbon
4.	β.-Myrcene	9.953	1.06	$C_{10}H_{16}$	Monoterpene
5.	α.-Phellandrene	10.591	4.64	$C_{10}H_{16}$	Monoterpene
6.	1,3-Cyclohexadiene	10.921	0.45	$C_{10}H_{14}$	Diene
7.	Cyclohexene	11.51	20.84	$C_{10}H_{16}$	Cycloalkene
8.	Bicyclo[3.1.0]hexane	11.695	1.93	$C_{10}H_{17}$	Cycloalkene
9.	Benzene	12.037	1.68	$C_{11}H_{14}$	Aromatic hydrocarbon
10.	1,4-Cyclohexadiene	12.453	1.08	$C_{11}H_{16}$	Diene
11.	Terpineol, Z-.β.-	13.072	0.37	$C_{10}H_{18}O$	Monoterpenoid alcohol
12.	1,6-Octadien-3-ol	13.918	0.35	$C_{10}H_{18}O$	Alcohol
13.	Terpineol, Z-.β.-	14.236	0.35	$C_{10}H_{18}O$	Alcohol
14.	Benzene	14.527	0.18	$C_{10}H_{18}O$	Alcohol
15.	2-Cyclohexen-1-ol	14.831	0.25	$C_{10}H_{18}O$	Alcohol
16.	2-Cyclohexen-1-ol	15.572	0.26	$C_{10}H_{18}O$	Alcohol
17.	3-Cyclohexen-1-ol	16.716	0.96	$C_{10}H_{18}O$	Alcohol
18.	(+)-α-Terpineol	17.329	0.32	$C_{10}H_{18}O$	Monoterpenoid alcohol
19.	2-Cyclohexen-1-ol	17.642	0.25	$C_{10}H_{18}O$	Alcohol
20.	Bicyclo[3.1.0]hexan-3-ol	18.021	0.25	$C_{10}H_{18}O$	Alcohol
21.	Cyclohexanone	18.487	0.15	$C_{11}H_{16}O$	Ketone
22.	Cyclohexene	18.941	0.82	$C_{11}H_{16}O$	Ketone
23.	α-Cubebene	19.168	0.42	$C_{15}H_{24}$	Terpene
24.	Benzaldehyde	19.78	0.48	$C_{11}H_{14}O$	Aromatic aldehyde
25.	Copaene	20.077	3.88	$C_{15}H_{24}$	Sesquiterpene
26.	1H-Cycloprop[e]azulene	20.663	1.01	$C_{15}H_{24}$	Polycyclic hydrocarbon

Peak No.	Compound name	RT (min)	Area (%)	Chemical formula	Chemical Class
27.	Benzene	20.957	0.51	$C_{11}H_{14}O$	Aromatic ether
28.	Tetradecane	21.241	0.21	$C_{14}H_{29}Cl$	Aliphatic halide
29.	Caryophyllene	21.889	13.58	$C_{15}H_{24}$	Sesquiterpene
30.	1,6,10-Dodecatriene	22.082	0.09	$C_{15}H_{24}$	Diene
31.	Pentadecane	22.224	0.02	$C_{16}H_{34}$	Alkane
32.	α-Caryophyllene	22.705	1.09	$C_{15}H_{24}$	Sesquiterpene
33.	GUAIENE	22.995	0.18	$C_{15}H_{24}$	Sesquiterpene
34.	1,3-Cyclohexadiene	23.365	0.42	$C_{13}H_{18}$	Cycloalkene
35.	Cyclohexene	23.599	5.23	$C_{14}H_{22}$	Cycloalkene
36.	Naphthalene	23.78	0.37	$C_{16}H_{26}$	Polycyclic aromatic hydrocarbon (PAHs)
37.	Naphthalene	24.415	1.68	$C_{16}H_{26}$	Polycyclic aromatic hydrocarbon (PAHs)
38.	γ-Elemene	25.54	0.31	$C_{15}H_{24}$	Sesquiterpene
39.	Dodecane	25.887	0.13	$C_{13}H_{28}$	Alkane
40.	Heptadecane	26.472	0.08	$C_{18}H_{38}$	Alkane
41.	Caryophyllene oxide	26.757	0.33	$C_{15}H_{24}O$	Terpene oxide
42.	12-Oxabicyclo[9.1.0]dodeca-3,7-diene	27.471	0.09	$C_{15}H_{22}O$	Bicyclic compound
43.	Naphthalenol	28.045	0.2	$C_{16}H_{26}O$	Alcohol
44.	7-Hexadecenal	28.3	0.07	$C_{16}H_{30}O$	Aldehyde

As shown in table 3 results also in line with Morshed *et al*. (2017) who observed the physicochemical characteristics and volatile composition of black pepper essential oil. They identified β-caryophyllene (19.12%), limonene (9.74%), and camphene (8.44%) as the primary volatile constituents determining its distinct aromatic and bioactive profile. Similar compounds also detected in this experiment too peak 2 for camphene and Peak number 29,32 and 41 multiple time detected with adduct (Table 3 and Table 4). Tran *et al*. (2019) reviewed on piperine as the primary functional alkaloid driving the spice's pungency. They also reported piperine content fluctuates significantly (typically 2% to 9%) depending on regional climate, origin and drying conditions but in our experiments Bicyclo[3.1.0]hexane, 4-methylene-1-(1-methylethyl) was reported with area % 23.21 with synonymous as sabinene and It has property of pungency. So our results indicated that Indian Black pepper pungency might be due to this compound. Sander *et al.* (2025) observed the differences between traditional ethanolic extraction methods and hydro distillation and reported the complexity depending on the choice of organic solvent or distillation method.

In conclusion, both the volatile and fixed oils may be varying due to extraction methods as well as solvent system used. (Sander *et al.* 2025 and Tran *et al*. 2019). Similarly, analytical platform and detector also shown varying in the compound detection too. Numerous reported reviews suggested these detected compounds had role in biological activities (Table 3 and Table 4)

Table 3 : Name of "key marker" bioactive compounds detected through GC MS

Crops	Name of important bioactive compounds detected	Uses define in literature	Reported by
Black pepper (*Piper nigrum* L.)	Piperine Sabinen, Caryophyllene, Caryophyllene oxide, 1,4-Cyclohexadiene, 1-methyl-4-(1-methylethyl)-	Spicy Anticancer Antioxidants bioavailability of a number of therapeutic drugs	Takooree *et al*(2019), Milenkovic and Stanojevic (2021).

Milenkovic and Stanojevic (2021) also reviewed that black pepper contains many substances such as terpenes, alkaloids, lignans, phenylpropanoids, etc., which are responsible for some of the most important biological activities: antioxidant, antimicrobial, anticancer, anti-inflammatory, analgesic, antipyretic, hepatoprotective, bio-enhancing and enzyme inhibitory activities.

Table 4 : Application of compounds detected in volatile oils of black pepper.

No.	Compound identified	Major application / biological significance	Citation
1	(1R)-2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene	use in fragrance, flavour and botanical bioactivity research.	Masyita*et al.,* 2022; De Sousa*et al.,* 2023
2	Camphene	antioxidant and antimicrobial activity.	Masyita*et al.,* 2022; De Sousa *et al.,* 2023
3	Bicyclo[3.1.0]hexane, 4-methylene-1-(1-methylethyl)-	Fragrance, flavour and plant volatile studies.	De Sousa *et al.,* 2023
4	β-Myrcene	flavour and aroma compound; antioxidant, anti-inflammatory, analgesic, anxiolytic and antimicrobial	Raut and Karuppayil, 2014; Masyita*et al.,* 2022)
5	α-Phellandrene	fragrance and flavour compounds also had antimicrobial, insecticidal, larvicidal, antinociceptive and antitumor activities; also has potential food and agricultural applications.	Radice *et al.,* 2022
6	1,3-Cyclohexadiene, 1-methyl-4-(1-methylethyl)-	application in flavour, fragrance and botanical-product formulations.	De Sousa *et al.,* 2023
7	Cyclohexene, 1-methyl-4-(1-methylethenyl)-, (S)-	use in flavour/fragrance formulations and phytochemical studies.	Masyita*et al.,* 2022
8	Bicyclo[3.1.0]hexane, 4-methylene-1-(1-methylethyl)-	fragrance and flavour	De Sousa *et al.,* 2023
9	Benzene, 1-methyl-4-(1-methylethyl)-(p-cymene)	Used as a fragrance/flavour-related volatile and studied as a component of antimicrobial and antioxidant essential-oil systems.	Masyita*et al.,* 2022; De Sousa *et al.,* 2023
10	1,4-Cyclohexadiene, 1-methyl-4-(1-methylethyl)-	Fragrance and phytochemical	De Sousa *et al.,* 2023

No.	Compound identified	Major application / biological significance	Citation
11	Z-β-Terpineol	Antimicrobial and antioxidant properties.	Masyita*et al.,* 2022; De Sousa*et al.,* 2023
12	1,6-Octadien-3-ol, 3,7-dimethyl-(Linalool)	perfumes, cosmetics, soaps and flavourings; antimicrobial, antioxidant, anti-inflammatory	Masyita*et al.,* 2022; De Sousa *et al.,* 2023
13	Z-β-Terpineol	Fragrance and flavour-	Masyita*et al.,* 2022
14	Benzene, 1-methyl-4-(1-methylethenyl)-	fragrance/flavour preparations and as a constituent of botanical volatile mixtures.	De Sousa*et al.,* 2023
15/16/17	2-Cyclohexen-1-ol, 1-methyl-4-(1-methylethyl)-, trans-	potential fragrance and phytochemical applications.	De Sousa*et al.,* 2023
18	(+)-α-Terpineol (p-menth-1-en-8-ol)	Fragrance, perfumes, cosmetics and antimicrobial, antioxidant and anti-inflammatory activities.	Masyita*et al.,* 2022; De Sousa*et al.,* 2023
19	2-Cyclohexen-1-ol, 3-methyl-6-(1-methylethyl)-, trans-	fragrance value and biological activity of essential oils.	De Sousa*et al.,* 2023
20	Bicyclo[3.1.0]hexan-3-ol, 4-methylene-1-(1-methylethyl)-, (1α,3α,5α)-	used as fragrance, flavour and botanical-product application.	De Sousa *et al.,* 2023
21	Cyclohexanone, 2-methyl-5-(1-methylethenyl)-	use in fragrance and flavour research.	De Sousa *et al.,* 2023
22	Cyclohexene, 4-ethenyl-4-methyl-3-(1-methylethenyl)-1-(1-methylethyl)-, (3R-trans)-	e useful as a chemotaxonomic marker of plant volatile profiles.	De Sousa *et al.,* 2023
23	α-Cubebene	potential antimicrobial and antioxidant relevance.	Semwal *et al.,* 2022
24	Benzaldehyde, 4-(1-methylethyl)-	important aroma	De Sousa *et al.,* 2023
25	Copaene	as a phytochemical/chemotaxonomic marker.	Da Trindade *et al.,* 2018
26	1H-Cycloprop[e]azulene, decahydro-1,1,7-trimethyl-4-methylene-...	contributes to essential-oil aroma	De Sousa*et al.,* 2023
27	Benzene, 1-methoxy-4-(1-propenyl)-(Anethole)	used in food flavouring, perfumes and cosmetic products.	Masyita*et al.,* 2022; De Sousa *et al.,* 2023
28	Tetradecane, 1-chloro-	Chlorinated hydrocarbon; when detected by GC–MS it should be interpreted cautiously because its occurrence may reflect environmental, analytical or contaminant sources rather than a characteristic plant metabolite.	De Sousa *et al.,* 2023
29	Caryophyllene (β-Caryophyllene)	extensively studied for antioxidant, antimicrobial, anti-inflammatory, analgesic, immunomodulatory and CB -receptor-related pharmacological properties.	Fidyt*et al.,* 2019; Hashiesh*et al.,* 2021

No.	Compound identified	Major application / biological significance	Citation
30	1,6,10-Dodecatriene, 7,11-dimethyl-3-methylene-, (E)-	potential value in fragrance and chemotaxonomic investigations.	De Sousa*et al.,* 2023
31	Pentadecane, 4-methyl-	Useful in chemical profiling of plant volatiles.	De Sousa *et al.,* 2023
32	α-Caryophyllene (α-Humulene)	in fragrance/flavour andanti-inflammatory, antioxidant and anticancer potential.	De Sousa *et al.,* 2023
33	trans-β-Guaiene	useful in fragrance	De Sousa *et al.,* 2023
34	1,3-Cyclohexadiene, 5-(1,5-dimethyl-4-hexenyl)-2-methyl-	chemotaxonomic marker.	De Sousa *et al.,* 2023
35	Cyclohexene, 1-methyl-4-(5-methyl-1-methylene-4-hexenyl	potential fragrance and phytochemical significance.	De Sousa *et al.,* 2023
36-37	Naphthalene, 1,2,4a,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-	chemotaxonomic &phytochemical marker.	De Sousa *et al.,* 2023
38	γ-Elemene	cytotoxic/anticancer activity of elemene-rich preparations.	De Sousa *et al.,* 2023
39	Dodecane, 2-methyl-	bioactive compound.	De Sousa *et al.,* 2023
40	Heptadecane, 3-methyl-	component of plant volatile	De Sousa*et al.,* 2023
41	Caryophyllene oxide	antimicrobial, antifungal, antioxidant and anticancer properties; also important in essential-oil and flavour/fragrance research.	De Sousa *et al.,* 2023; Masyita*et al.,* 2022
42	12-Oxabicyclo[9.1.0]dodeca-3,7-diene, 1,5,5,8-tetramethyl-...	aroma	De Sousa*et al.,* 2023
43	1-Naphthalenol	Fragrance	De Sousa *et al.,* 2023
44	7-Hexadecenal, (Z)-	flavour/fragrance	De Sousa *et al.,* 2023

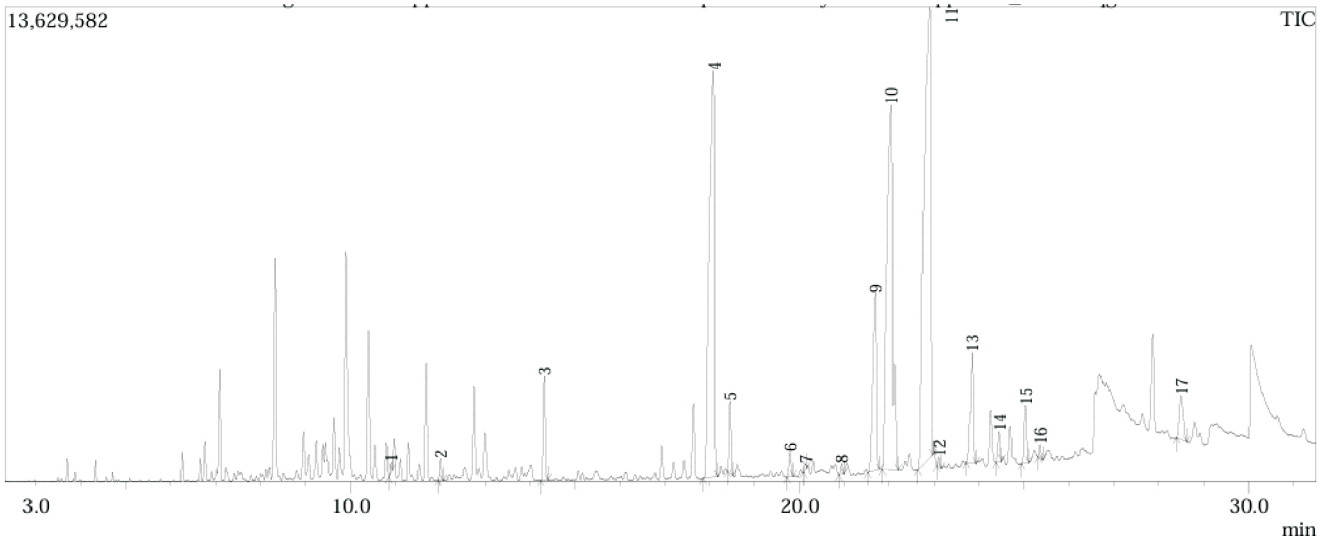


Figure : Chromatogram on FAME of black pepper oil

Table.5 Fatty acids profiling of fixed oils extracts through hexane in black pepper

Peak	R.Time	Area%	Height%	A/H	Name
1	10.907	0.16	0.52	2.21	Dodecanoic acid
2	12.013	0.47	1.13	3.05	--
3	14.318	2.38	5.44	3.19	Methyl tetradecanoate
4	18.088	22.6	21.24	7.76	Hexadecanoic
5	18.456	1.66	3.79	3.2	9-Hexadecenoic acid
6	19.788	0.61	1.31	3.38	Heptadecanoic acid
7	20.126	0.12	0.31	2.67	--
8	20.934	0.24	0.57	3.05	9,12-Octadecadienoic acid
9	21.688	7.4	9.27	5.82	Octadecanoic acid
10	22.04	23.1	19.07	8.84	9-Octadecenoic acid
11	22.901	32.91	23.53	10.2	9,12-Octadecadienoic acid
12	23.105	0.2	0.49	2.95	7-Tetradecenal
13	23.853	3.7	5.77	4.68	9,12,15-Octadecatrienoic acid
14	24.441	0.69	1.59	3.19	9,12-Octadecadienoic
15	25.027	1.55	3	3.76	Eicosanoic acid
16	25.356	0.26	0.69	2.79	11-Eicosenoic acid
17	28.493	1.95	2.28	6.23	Docosanoic acid

The analysis of the area percentages revealed that the minimum value was 0.12%, belonging to Cyclopropaneoctanoic acid, a 18-carbon MUFA compound. On the other hand, the maximum value was 32.91% attributed to 9,12-Octadecadienoic acid, an 18-carbon PUFA compound (Table.6). The fixed oil extracted from black pepper contains a diverse range of fatty acids, each contributing to the overall composition and properties of the oil. The analysis of the fatty acids presents in the extract revealed a variety of saturated and unsaturated fatty acids, with different carbon chain lengths and functionalities.

In the analysis, the SFA class of fatty acids accounted for 36.65% of the total area, while MUFA made up 25.81%. PUFA contributed the highest percentage at 37.54%. The ratio of MUFA/PUFA was calculated as 0.69. Additionally, the O/L group represented 6.24% of the total area, and the UnsFA/SFA ratio was found to be 38.24%. The Data on fixed oils with their FAME compounds of *Piper nigrum L* are presented in Table5 and figure2.In the extracted oil, saturated fatty acids such as Palmitic acid and Stearic acid were prominent components, providing stability and structure to the oil. These saturated fatty acids contribute to the overall texture and shelf-life of the oil. Additionally, unsaturated fatty acids were found in abundance in the fixed oil of black pepper. Oleic acid, a monounsaturated fatty acid, was identified as a significant component, offering health benefits and flavour characteristics to the oil. Furthermore, the presence of Linoleic acid, a polyunsaturated fatty acid, adds nutritional value and oxidative stability to the oil.

Table 6. Class of fatty acids presence in the extracts fixed oil of black pepper

Peak#	R.Time	Area%	Name	Chemical Formula	Carbon Number	Class
1	10.907	0.16	Dodecanoic acid	$C_{12}H_{24}O_2$	12	SFA
2	12.013	0.47	Tridecanoic acid	$C_7H_{12}O_2$	7	MUFA
3	14.318	2.38	Methyl tetradecanoate	$C_{15}H_{30}O_2$	14	SFA
4	18.088	22.6	Hexadecanoic acid	$C_{16}H_{32}O_2$	16	SFA
5	18.456	1.66	9-Hexadecenoic acid	$C_{16}H_{32}O_2$	16	MUFA
6	19.788	0.61	Heptadecanoic acid	$C_{17}H_{34}O_2$	17	SFA
7	20.126	0.12	Cyclopropaneoctanoic acid	$C_{18}H_{34}O_2$	18	MUFA
8	20.934	0.24	9,12-Octadecadienoic acid	$C_{18}H_{32}O_2$	18	PUFA
9	21.688	7.4	Octadecanoic acid	$C_{18}H_{36}O_2$	18	SFA
10	22.04	23.1	9-Octadecenoic acid	$C_{18}H_{34}O_2$	18	MUFA
11	22.901	32.91	9,12-Octadecadienoic acid	$C_{18}H_{32}O_2$	18	PUFA
12	23.105	0.2	7-Tetradecenal, (Z)-	$C_{14}H_{26}O_2$	14	MUFA
13	23.853	3.7	9,12,15-Octadecatrienoic acid	$C_{18}H_{30}O_2$	18	PUFA
14	24.441	0.69	9,12-Octadecadienoic acid	$C_{18}H_{30}O_2$	18	PUFA
15	25.027	1.55	Eicosanoic acid	$C_{20}H_{40}O_2$	20	SFA
16	25.356	0.26	11-Eicosenoic acid	$C_{20}H_{38}O_2$	20	MUFA
17	28.493	1.95	Docosanoic acid	$C_{22}H_{44}O_2$	22	SFA
		99.37	Class of fatty acids	**Total Area %**		
			SFA	36.65		
			MUFA	25.81		
			PUFA	37.54		
			MUFA/PUFA	0.69		
			O/L	6.24		
			UnsFA/SFA	38.24		

CONCLUSION

The fatty acid profile of the fixed oil extracted from black pepper signifies a well-balanced composition that combines essential saturated and unsaturated fatty acids. This composition not only influences the sensory attributes of the oil but also contributes to its nutritional value and potential health benefits. In the analysis, the SFA class of fatty acids accounted for 36.65% of the total area, while MUFA made up 25.81%. PUFA contributed the highest percentage at 37.54%. The ratio of MUFA/PUFA was calculated as 0.69. Additionally, the O/L group represented 6.24% of the total area, and the UnsFA/SFA ratio was found to be 38.24%. The Fixed and Volatile oil compounds of *Piper nigrum* L. (Black pepper) composed of 17 fatty acids and 44 compounds detected through GCMS analysis.

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