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Phytochemical screening and pharmacological activity of Bush pepper (*Piper nigrum* L.) through gas chromatography and mass spectroscopy technique

P. Vasantharaj*, D. Rajakumar*◆, A. Jaya Jasmine, M. Hemalatha*, M. Gomathy** and K. Sivakumar****

* *Department of Agronomy, V.O.C. Agricultural College and Research Institute, Tamil Nadu Agricultural University, Killikulam, Thoothukudi-628252, Tamil Nadu, India*

** *Horticultural Research Station, Tamil Nadu Agricultural University, Pechiparai, Kanniyakumari-629161, Tamil Nadu, India*

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Abstract

This study explores the phytochemical compounds and their pharmacological properties in Bush pepper (*Piper nigrum* L.) berries of two distinct agroforestry systems: A rubber-based system (*Hevea brasiliensis*) and a terminalia-based system (*Terminalia elliptica*). The essential oil content was found to be slightly higher in the rubber-based system (3.48%) compared to the terminalia-based system (3.44%). Similarly, oleoresin content was greater in the rubber-based system (12.17%) than in the terminalia-based system (11.90%). Gas chromatography-mass spectrometry analysis revealed a diverse phytochemical profile in the essential oils, with significant constituents identified such as piperine as the main component. Other identified key constituents include monoterpenes and sesquiterpenes such as β-caryophyllene, limonene, phytol, pinene and pentadecane. However, few uncommon compounds such as spiro [5.5] undecane and pempidine in the rubber-based system, 2-adamantanone and 1,3-phenylene, bis (3-phenylpropenoate) in the terminalia-based system occupied significant proportions in the essential oil content. Variations in essential oil composition are influenced by agroforestry systems and related factors affecting secondary metabolite synthesis. The diverse phytochemical profiles of *P. nigrum* reveal its significant bioactive properties and potential in phytomedicine. The compound's antimicrobial, antifungal and cytotoxic activities suggest promising therapeutic applications. As interest in natural products continues to grow, the pharmacological potential of *P. nigrum* presents valuable opportunities for drug development and nutraceutical applications. The integration of agroforestry practices can optimize the yield and quality of these bioactive compounds, paving the way for innovative therapeutic solutions.

1. Introduction

Plants have historically been essential sources for numerous chemicals used in pharmaceuticals, fragrances, food colourings and flavours, especially in India. Plant-derived molecules have proven highly promising in therapeutic applications. Spices and herbs are rich in natural antioxidants, vital for chemoprevention of diseases and the ageing process, offering significant health benefits (Singh *et al.*, 2012). Black pepper (*Piper nigrum* L.), a commercially important spice belonging to Piperaceae family. Bush pepper is novel technique developed by Indian Institute of Spices Research (IISR), Calicut, in order to cultivate pepper in the form of bush instead of vine. *P. nigrum* is indigenous to South India specifically to the evergreen forests of the Western Ghats. More than twenty-five countries, including India, Indonesia and Malaysia, cultivate black pepper. It is often referred to as the 'King of Spices' and 'Black gold' for its contribution in the country's export sector and in international trade (Gayathri *et al.*, 2023).

Corresponding author: Dr. D. Rajakumar
Associate Professor, Department of Agronomy, V.O.C. Agricultural College and Research Institute, Tamil Nadu Agricultural University, Killikulam, Thoothukudi-628252, Tamil Nadu, India
E-mail: rajakumar.d@tnau.ac.in
Tel.: +91-9597650495

Besides its major role in international trade and as a culinary spice, black pepper has been greatly recognised for the potential medicinal uses of its dried berries (Kumar *et al.*, 2021). In traditional medicine, black pepper is highly valued for its gastrointestinal effects, such as increasing appetite. It is also used to treat coughs, colds, throat diseases, dysentery, stomach-aches, worms, piles, dyspnea and intermittent fever (Gülçin, 2005). Remarkable pharmacological properties of black pepper include antioxidant, antimicrobial, antidiarrhoeal, analgesic, antipyretic, anti-inflammatory, anti-convulsant, antimutagenic, neuroprotective, hypoglycemic and hypolipidemic agent (Bhavsar *et al.*, 2022; Takooree *et al.*, 2019). Furthermore, black pepper functions as an anticancer, diuretic and aphrodisiac agent. It also serves as a blood purifier, enhances nutrient bioavailability, accelerates lipid metabolism and stimulates both the gastrointestinal and central nervous systems (Joshi *et al.*, 2018).

The quality of pepper berries is determined by its biochemical compounds. Its pungency is primarily attributed to the alkaloid piperine, while its aroma and flavour come from compounds of volatile oils. Piperine has been shown to enhance oral bioavailability by inhibiting various metabolizing enzymes, thereby improving the therapeutic effectiveness of many medications, vaccines and minerals (Kale *et al.*, 2021; Mehrotra, 2021). Furthermore, piperidine and piperanine in *P. nigrum* have shown significant potential in combatting COVID-19, highlighting their promising role in the fight against this global pandemic (Rana and Chauhan, 2022). Black pepper seeds from South India primarily exhibit a β-caryophyllene-dominant essential oil profile, with notable amounts of limonene, sabinene, α-pinene, β-bisabolene, α-copaene, α-cadinol, α-thujene and α-humulene. In contrast, pepper leaves are characterized by a high concentration of nerolidol, followed by α-pinene and β-caryophyllene (Ashokkumar *et al.*, 2021).The diversity of active compounds in black pepper can vary significantly due to factors such as the species, environmental conditions, cultivation practices, post-harvest management, oil extraction methods and even the form of pepper being cultivated (*i.e.,* vine or bush) (Said-Al Ahl *et al.*, 2024). Bush pepper is raised from fruiting branches of vine pepper and produces spikes from all its branches yielding berries throughout the year (Gayathri *et al*., 2023). *P. nigrum* thrives well under partial shade, yielding more effectively. In addition to effective space utilization, a convenient agroforestry system provides optimum shade and enhances pepper growth by improving root development and nutrient uptake (Riyaz *et al.*, 2023). Fertilizer application also have significant impact on the quality and yield of spices and medicinal plants (Chauhan *et al.*, 2023; Kianimanesh *et al.*, 2021). These practices, together with other influencing factors, can lead to notable changes in the chemical profile, particularly in the essential oils (Megat Ahmad Azman *et al.*, 2020). This study aimed to identify the phytochemical compounds in the essential oil extracted from berries of Bush pepper plant grown under the two distinct agroforestry systems using GC-MS analysis.

2. Materials and Methods

2.1 Sample collection

Matured berries, along with intact spikes, were collected from well-established Bush pepper plants (Panniyur 1 variety), cultivated under two distinct agroforestry systems: A rubber-based system (*Hevea brasiliensis*) and terminalia-based system (*Terminalia elliptica*) at the Horticultural Research Station, Tamil Nadu Agricultural University (TNAU), Pechiparai, Kanyakumari district.The berries were selectively harvested from block of high-yielding plants that had been consistently supplied with sufficient quantity of fertilizers along with arbuscular mycorrhizal fungi. Berries harvested from both agroforestry systems were subjected to subsequent oil extraction and phytochemical analysis.

The plant specimen was scientifically identified and authenticated by Dr. M. Johnson, Curator, Centre for Plant Biotechnology, Department of Botany, St. Xavier's College (Autonomous), Palayamkottai, Tirunelveli, Tamil Nadu. Also, herbarium prepared was deposited in Centre for Plant Biotechnology Herbarium (Indexed on 2022), Department of Botany, St. Xavier's College (Autonomous), Palayamkottai with Voucher Specimen Number SXC-CPBH-5207 for future reference.

2.2 Extraction of essential oil

The extraction and analysis of essential oil content in *P. nigrum* berries was done using the procedures described by Tran *et al.* (2019). Harvested berries were completely shade dried at room temperature around 25°C. 20 g of dried *P. nigrum* berry powder, mixed with glass beads so as to prevent foaming, were placed in a 1000 ml volumetric flask and connected to a Clevenger apparatus. The mixture was subjected to heating using a thermostat-controlled heating mantle. Distillation was performed at 90°C until the mixture began to boil, after which the temperature was reduced to 70°C and maintained for 3 hours. The extraction was considered to commence upon the appearance of the first drop of essential oil. Following the distillation, the distillate was allowed to cool to room temperature to facilitate phase separation. The essential oil was then isolated, dehydrated with sodium sulphate (Na_2SO_4) and its content was quantified using the standard formula:

$$\text{Essential oil content (\%)} = \frac{\text{Volume of oil obtained (ml)}}{\text{Weight of dried sample used (g)}} \times 100$$

The essential oil content was assessed for three samples from each agroforestry system and the mean values were represented in percentage.

2.3 Preparation protocol for crude extract

Harvested berries of *P. nigrum* were thoroughly cleaned and dried in the shade. After drying, they were stored in airtight containers to prevent moisture absorption and kept at room temperature until further processing. A powdered sample of 30 g was soaked in 100 ml of methanol for 16 h using a rotary shaker. The mixture was then filtered through Whatman No. 1 filter paper to separate the extract. The filtrate was further purified by passing it through sodium sulphate to remove any residual moisture before being used for subsequent phytochemical analysis (Mohammed *et al.*, 2016).

2.4 Oleoresin content

To assess the oleoresin content, the methanol from the filtered crude extract was removed by evaporating it under reduced pressure using a rotary evaporator, leaving behind the oleoresin. The weight of the oleoresin was then measured. This weight was compared to the initial weight of the powdered plant material to determine the oleoresin content using the formula:

Oleoresin content (%)

$$= \frac{\text{Weight of oleoresin obtained (g)}}{\text{Weight of powdered sample taken (g)}} \times 100$$

2.5 GC-MS analysis

The essential oil was diluted with n-hexane at a ratio of 1:50. About 1 µl aliquot of this diluted sample was introduced into an Agilent 7890A GC *via* an automatic injector, coupled with an Agilent 5975C MS. The system utilized an HP-5MS capillary column (5% phenyl methyl siloxane) and operated with an electron ionization source at 70 eV. Helium was employed as the carrier gas with a flow rate of 1 ml/min. The injector and ion source were maintained at 250°C and 280°C, respectively. The GC oven temperature program was as follows: 60°C for 2 min, ramped from 60°C to 210°C at 5°C/min, held at 210°C for 0 min, ramped from 210°C to 280°C at 10°C/min and finally held at 280°C for 1 min.

2.6 Identification of GC-MS detected phytochemicals

Mass spectra from the GC-MS data were compared with spectra of existing records from the Wiley and National Institute of Standards and Technology (NIST) database,which covers over 90,000 compounds and then analysed the retention indices of screened bioactive compounds with available data.

3. Results

3.1 Essential oil content

A notable variation in essential oil concentration of *P. nigrum* berries were observed between the two agroforestry systems (Figure 1). The essential oil content is marginally greater in the rubber-based agroforestry system (3.48%) compared to the terminalia-based agroforestry system (3.44%).This represents a 1.16% increase in the rubber-based system.

3.2 Oleoresin content

A significant variation was recorded in the oleoresin content of *P. nigrum* berries within the two agroforestry systems (Figure 2). In the rubber-based agroforestry system, the oleoresin content of *P. nigrum* berries is slightly higher (12.17%) compared to the terminalia-based agroforestry system (11.90%). This results in a 2.28% increase in oleoresin content in the rubber-based system relative to the terminalia-based system.

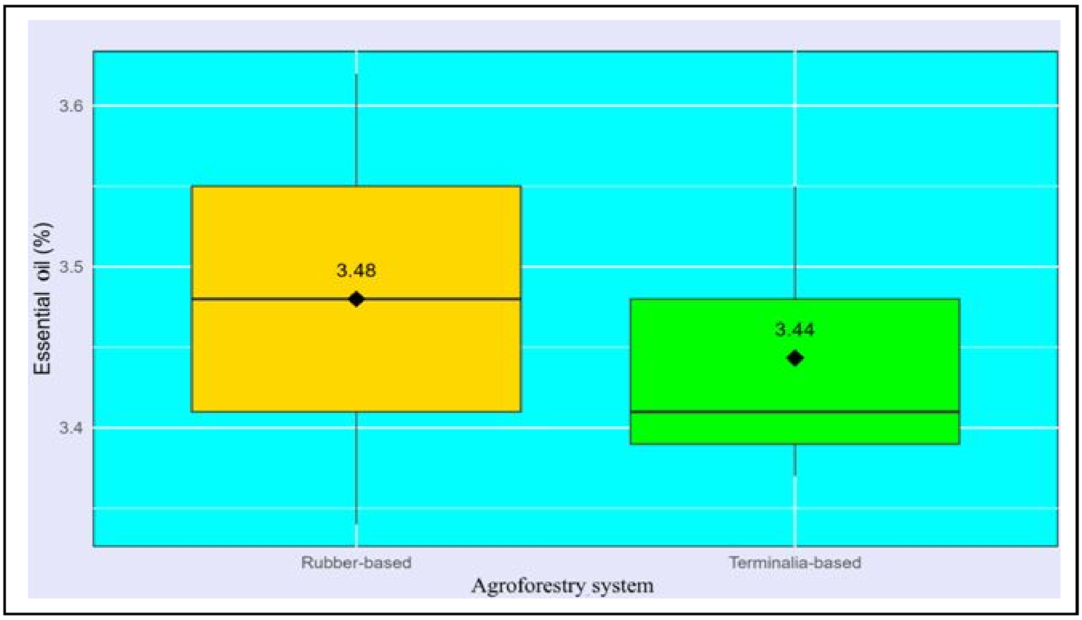

Figure 1: Essential oil concentration in *P. nigrum* berries under both agroforestry systems.

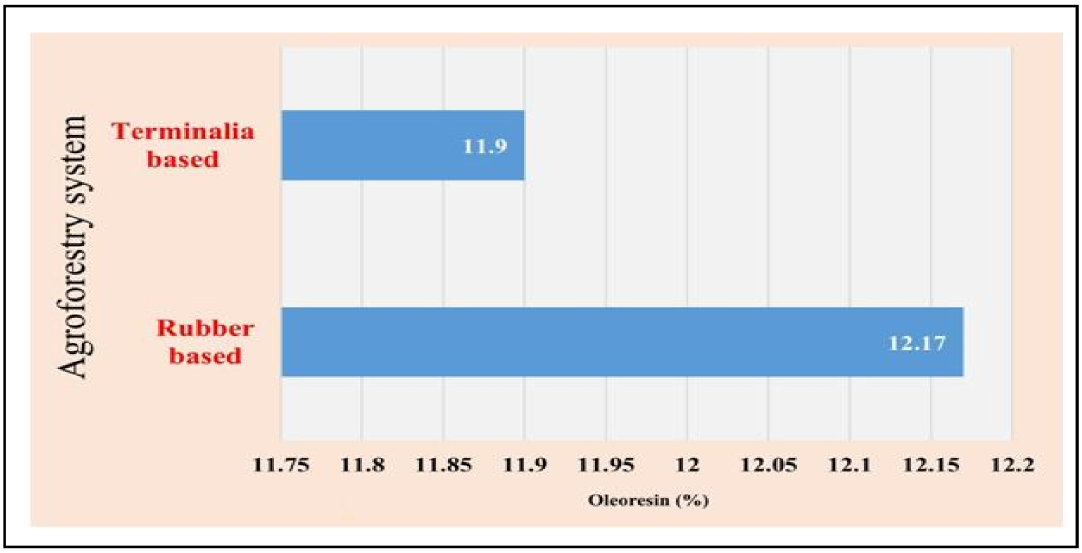

Figure 2: Oleoresin content in *P. nigrum* berries within each agroforestry system.

3.3 Phytochemical profiling through GC-MS

The essential oil was extracted from *P. nigrum* berries through steam distillation. GC-MS analysis was performed to identify the bioactive compounds present in the essential oil extracted from berries of both agroforestry systems.

3.3.1 Rubber-based agroforestry system

GC-MS analysis revealed that the essential oil contains a variety of phytochemicals and recorded 60 peaks as shown in the chromatogram (Figure 3). Major phytochemicals screened in essential oil includes piperine, 1,2-benzenediol, O-(2-methoxybenzoyl)-O'-

(propargyloxycarbonyl)-, spiro [5.5] undecane, caryophyllene, trans-cinnamoylimidazole and pempidine with peak area of 34.19%, 13.38%, 8.06%, 3.6%, 3.11% and 2.66%, respectively. These compounds are significant contributors to the oil's profile, highlighting the richness of its chemical composition. An overview of the major phytochemical compounds identified, including their retention time, peak area (%) and molecular formula, emphasizing those that constitute significant proportions is given in Table 1.

Table 1: Various metabolites in essential oil of *P. nigrum* berries, as identified by GC-MS technique (Rubber-based agroforestry system)

S. No.	Compounds	Retention time (min)	Peak area (%)
1	Piperine	19.129	34.19
2	1,2-Benzenediol, O-(2-methoxybenzoyl)-O'-(propargyloxycarbonyl)-	17.307	13.38
3	Spiro[5.5]undecane	17.952	8.06
4	Caryophyllene	9.297	3.60
5	N-trans-cinnamoylimidazole	19.418	3.11
6	Pempidine	20.996	2.66
7	2-Propenoic acid, 3-(4-methoxyphenyl)-, ethyl ester	11.886	2.05
8	Caranone, trans-4-	19.341	2.01
9	δ-Cadinol	11.13	1.63
10	3-Ethoxy-4-methoxybenzaldehyde	13.119	1.62
11	Limonene	5.365	1.56
12	L-β-Pinene	4.831	1.33
13	Copaene	10.086	1.30
14	1H-Pyrazole-3-carboxylic acid (methyl)(1,2,2,6,6-pentamethylpiperidin-4-yl)amide	18.852	1.16
15	β-Bisabolene	9.942	1.07
16	(E)-ethyl cinnamate	9.608	0.98
17	5,7-Dihydroxy-4-methylcoumarin	19.707	0.97
18	Thiazole, 4-ethyl-2-propyl-	18.385	0.91
19	Methyl p-anisate	17.496	0.73
20	1R-α-Pinene	4.331	0.64
21	Sabinene	4.753	0.62
22	p-Vinylbenzohydrazide	20.107	0.57
23	4-Methyldaphnetin	14.508	0.52
24	Spathulenol	10.986	0.48
25	p-Cyclohexylbenzoic acid	17.052	0.46
26	1-Iododec-1-yne	17.819	0.44
27	n-Pentadecane	9.808	0.34
28	Dihydrofurano(3,2-f)coumaranone	11.264	0.31
29	Dodecahydropyrido[1,2-b]isoquinolin-6-one	18.163	0.30
30	Methyl-1-adamantaneacetamide	18.518	0.26
31	Phytol	14.197	0.18
32	Piperonal	8.497	0.15
33	Piperidine, 1-cinnamoyl-	14.374	0.13

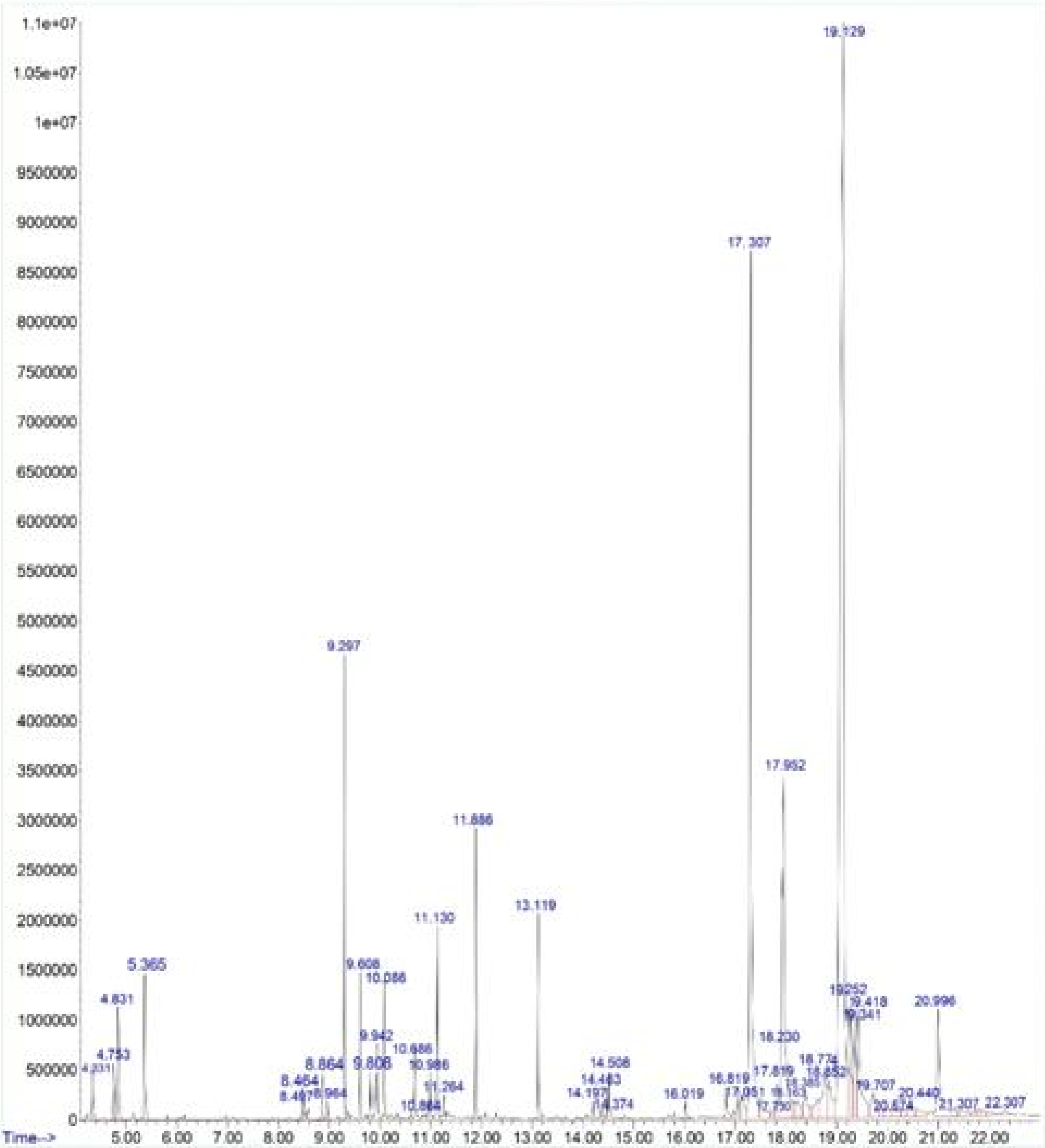

Figure 3: GC-MS chromatogram of essential oil obtained from berries of *P. nigrum* grown in rubber-based agroforestry system.

3.3.2 Terminalia-based agroforestry system

Major bioactive compounds present in the essential oil and information on these compounds, including their retention times, peak areas (%) and molecular formulas are presented in Table 2. The most common phytochemical constituents found here in the essential oil of *P. nigrum* berries were piperine, 2-adamantanone, 1,6-anhydro-4-(3,4-methylenedioxyphenylmethylamino)-2-O-tosyl-4-deoxy-b-d-glucopyranose, caryophyllene and 1,3-Phenylene, bis(3-phenylpropenoate) with retention time of 19.129, 17.941, 17.296, 9.297 and 19.418 min, respectively. The volatile profile of the essential oil of berries from this system is shown in the GC-MS chromatogram illustrated in Figure 4.

Table 2: Phytochemicals in essential oil of *P. nigrum* berries, as identified by GC-MS technique (Terminalia-based agroforestry system)

S. No.	Compounds	Retention time (min)	Peak area (%)
1	Piperine	19.129	32.64
2	1,6-Anhydro-4-(3,4-methylenedioxyphenylmethylamino)-2-O-tosyl-4-deoxy-b-d-glucopyranose	17.296	13.39
3	2-Adamantanone	17.941	7.94
4	Caryophyllene	9.297	5.02
5	1,3-Phenylene, bis(3-phenylpropenoate)	19.418	3.28
6	1,3,5-Triazin-2(1H)-one, 4,6-diamino-	20.996	2.44
7	Limonene	5.365	2.41
8	Caranone	19.341	1.95
9	Pulegone	19.252	1.88
10	L-β-Pinene	4.831	1.86
11	Copaene	11.13	1.78
12	γ-Muurolene	10.086	1.44
13	2-Thujene	4.754	1.1
14	β-Bisabolene	9.942	1.1
15	Piperidine, 1-cinnamoyl-	14.375	1.1
16	Ethyl p-methoxycinnamate	11.886	0.94
17	δ-Elemene	8.464	0.84
18	2-Methoxybenzoic acid, 2,4,6-trichlorophenyl ester	17.119	0.8
19	Benzoic acid, 4-hydroxy-, n-heptylester	17.496	0.7
20	Androst-2-ene-1,17-diol,4-methyl-(1α,4α,5β)	19.707	0.7
21	9-Hexadecenoic acid, pyrrolidide	20.44	0.58
22	α-Cubenene	8.864	0.54
23	Thiazole, 4-ethyl-2-propyl-	18.385	0.54
24	(E)-ethyl cinnamate	9.608	0.53
25	(2E,4E,10E)-11-(1,3-benzodioxol-5-yl)-N-isobutyl-2,4,10-undecatrienamide	18.23	0.41
26	3,4-Methylenedioxyphenylacetonitrile	20.118	0.39
27	2-Amino-7-chloroquinoxaline	14.463	0.34
28	Pyrimidin-4-ol, 6-methyl-2-(1-piperidyl)-	18.163	0.28
29	Aromadendrene	10.986	0.26
30	4-Methyldaphnetin	14.508	0.26
31	Benzimidazole, 2-(1H-1,2,4-triazol-5-ylthiomethyl)-	15.797	0.23
32	β-Tocopherol	19.985	0.23
33	Phytol	14.197	0.22
34	Calarene (β-gurjunene)	8.964	0.21
35	n-Pentadecane	9.808	0.21
36	9-Methoxybicyclo[6.1.0]nona-2,4,6triene	17.052	0.21
37	Terephthalic acid, di(2-ethylhexyl) ester	17.73	0.21
38	n-Hexadecanoic	13.197	0.18

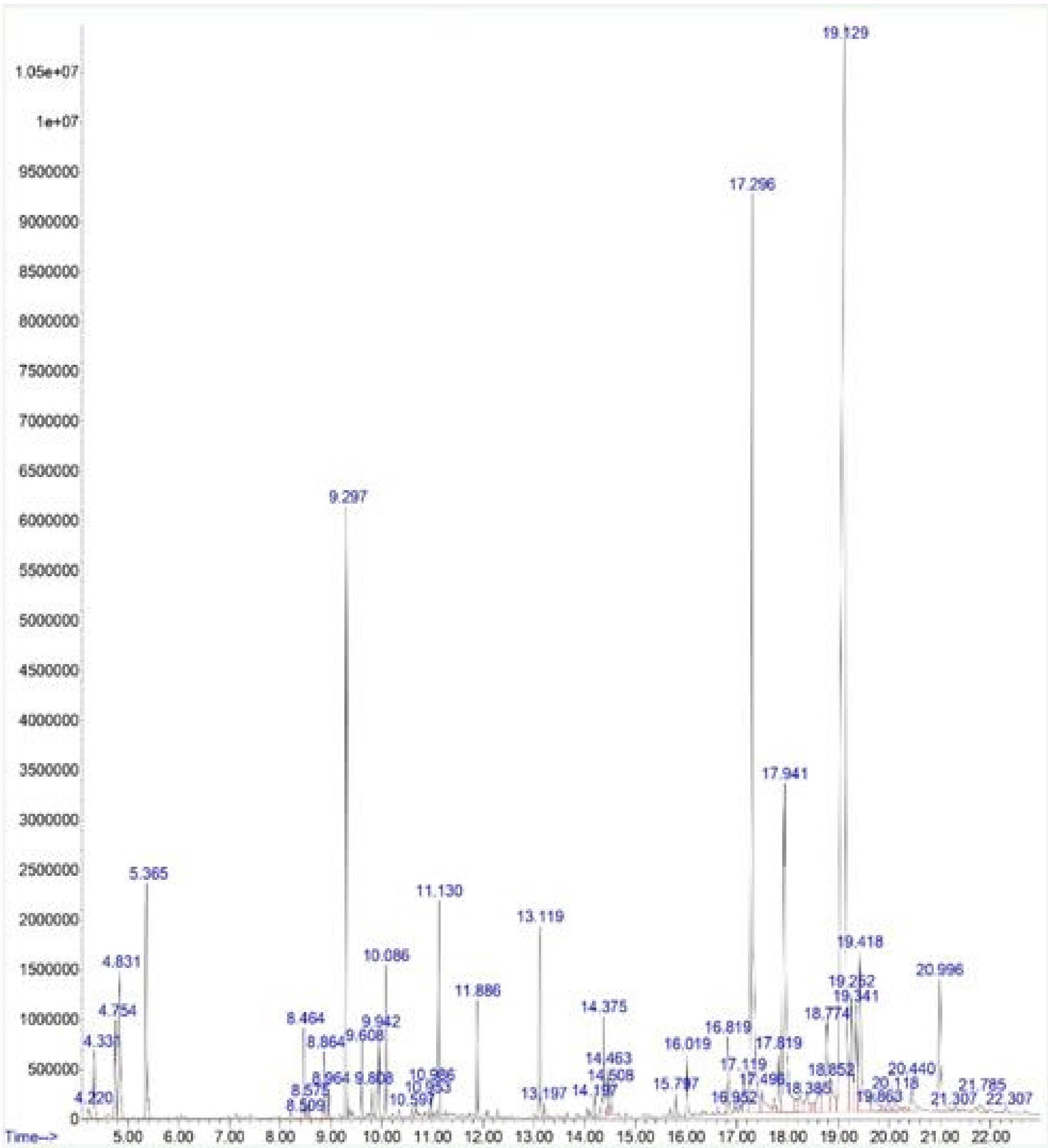

Figure 4: GC-MS chromatogram of essential oil obtained from berries of *P. nigrum* grown in terminalia-based agroforestry system.

Several compounds are commonly found in *P. nigrum* berries from both agroforestry systems, highlighting the fundamental constituents of essential oils. Many of these compounds exhibit valuable biological properties. Pharmacological properties of these phytochemicals identified in the essential oil of *P. nigrum*, as analysed *via* GC-MS, are included in Table 3.

Table 3: Pharmacological properties and chemical profile of bioactive compounds identified in both agroforestry system

Compound name	Molecular weight (g/mol)	Compound nature	Pharmacological properties	Reference
(E)-ethyl cinnamate	176.21	Ester	Antimicrobial, anti-inflammatory, antidiabetic, anticancer, neuroprotective	Pandey *et al.*, 2020
Caranone	150.22	Ketone	Antioxidant, antimicrobial	Noumi *et al.*, 2023
Caryophyllene	204.35	Sesquiterpene	Antioxidant, anticancer, cardioprotective, hepatoprotective, anti-inflammatory, nephroprotective, gastroprotective, antimicrobial, immunomodulator, wound healing	Arsana *et al.*, 2022
Copaene	204.35	Sesquiterpene	Antioxidant, antimicrobial, antifungal	Türkez *et al.*, 2014
Limonene	136.24	Monoterpene	Antibacterial, anticancer, analgesic, neuroprotection, antioxidant, anti-inflammatory	Chen *et al.*, 2024
n-Pentadecane	212.41	Alkane	Anti-inflammatory, analgesic, antipyretic	Okechukwu, 2020
Phytol	286.47	Terpene alcohol	Anti-diarrheal, antihyperalgesic, antiarthritic, anti-inflammatory, hepatoprotective, antioxidant	Carvalho *et al.*, 2020
Pinene	136.23	Monoterpene	Anticoagulant, anti-inflammatory, antimalarial, antimicrobial, antioxidant, antitumor, analgesic	Park *et al.*, 2021
Piperidine	85.13	Amine	Alzheimer's disease therapy drug, anticancer agents, antibiotics, analgesics, antipsychotics, antioxidants	Frolov and Vereshchagin, 2023
Piperine	285.34	Alkaloid	Antibacterial, anticancer, antidepressant, hepatoprotective, antioxidant, antidiabetic, antidiarrheal, neuroprotective, anticonvulsant, anti-inflammatory, antipyretic	Quijia *et al.*, 2021
β-Bisabolene	204.35	Sesquiterpene	Antibacterial, anti-inflammatory	Shu *et al.*, 2021

4. Discussion

The rubber-based agroforestry system was found to have a slight advantage over the terminalia-based system regarding oleoresin and essential oil yields. Similarly, the findings on *Ocimum* spp. in silvi-medicinal systems (Suvera *et al.*, 2015) and volatiles of *Pandanus amaryllifolius* in rubber intercropping (Zhang *et al.*, 2023) align with the advantages of rubber-based agroforestry systems in producing higher oleoresin and essential oil yields. In another aspect, Bush pepper plants supplied with a combination of fertilizer and arbuscular mycorrhizal fungi may have exhibited higher percentages of oil and oleoresin.This is due to improved nutrient uptake facilitated by mycorrhizal symbiosis, which stimulates secondary metabolite production, including essential oils. Arbuscular mycorrhizal fungi also boost the activity of key enzymes like phenylalanine ammonia-lyase and lipoxygenase (da Trindade *et al.*, 2019), vital for synthesizing phenolic and terpenoids components having broader pharmacological properties such as antioxidant, anti-inflammatory and antimicrobial effects.

GC-MS is a robust and precise technique for analysing a broad spectrum of metabolites with pharmacological significance, including sugars, organic acids, polyols and various phenolic as well as cyclic compounds. Phytochemical screening on the essential oil of *P. nigrum* grown under two distinct agroforestry systems showed the complex blend of various compounds, with piperine as the principal constituent in both cases, while other bioactive compounds in trace amounts. Dominant constituents identified in essential oil of *P. nigrum* are monoterpene and sesquiterpene hydrocarbons, such as β-caryophyllene, limonene, phytol, pinene and pentadecane which is in accordance with the findings of Arsana *et al.* (2022) and Dosoky *et al.* (2019). However, several novel bioactive compounds reported as minor constituents in previous studies, were found to constitute major proportions of the essential oil in this study. Evidently, these include 1,2-benzenediol, O-(2-methoxybenzoyl)-O'-(propargyloxy carbonyl)- and spiro [5.5] undecane under the rubber-based agroforestry system and 1,6-anhydro-4-(3,4-methylenedioxy phenylmethylamino)-2-O-tosyl-4-deoxy-b-d-glucopyranose, 1,3-phenylene, bis (3-phenylpropenoate) and 2-adamantanone in the terminalia-based agroforestry system. These compounds have been screened, identified and reported in various other herbal plants through GC-MS analysis. Those compounds along with their associated biological activities are discussed here.

The bioactive compound 1,2-benzenediol, O-(2-methoxybenzoyl)-O'-(propargyloxycarbonyl) is a derivative of catechol found in various plants. The production of catechol and its derivatives is often part of the plant's defence response (Liu *et al.*, 2007) against the competing nature of rubber trees with *P. nigrum*. Its pharmacological properties include antioxidant, antimicrobial and anticancer (Kim *et al.*, 2019).

Spiro [5.5] undecane also have antimicrobial and antitumor properties. It was observed in plants at elevated levels with application of nutrient at higher concentrations (Ahmed *et al.*, 2012). The compound 1,3-phenylene, bis (3-phenylpropenoate) that have antioxidant, antibacterial, antiplasmodial and anticancer properties (Wiraswati *et al.*, 2023) is produced in significant proportion due to the influence of shade provided by terminalia, as similar to the production of the same compound in soybean grown under tea (Duan *et al.*, 2021).

The phytochemicals found in relatively minimal proportion that screened in the essential oil of *P. nigrum* also constitute various applications in pharmacological and drug industry for their valuable therapeutic properties. Among them, pulegone is an important monoterpene with molecular weight of 152.24 g/mol. Its pharmacological properties include antimicrobial, antipyretic, convulsant, antifeedent, antiparasitical and antioxidant (Božoviæ and Ragno, 2017). Besides valuable pharmacological properties, it possesses a pleasant aroma reminiscent of peppermint and camphor, making it a valuable compound in the flavouring and fragrance industries (Amalich *et al.*, 2016). Another valuable monoterpene sabinene having molecular weight of 136.24 g/mol, is known for its antioxidant properties and used in aromatherapy. Besides, it was reported for antifungal and anti-inflammatory properties by Wang *et al.* (2021). Spathulenol**,** a sesquiterpene alcohol with molecular weight of 204.35 g/mol, is found in the essential oil of *Psidium guineense* (do Nascimento *et al.*, 2018) and was reported to effective against the ovarian cancer cell line, with a GI_{50} value of 0.89 μg/ml.This compound also possesses anticancer, antimicrobial and anti-inflammatory properties (Paksoy *et al.*, 2016). δ-Cadinolis also a well-known sesquiterpene alcohol found in various essential oils and plant extracts such as wood of *Cedrelopsis grevei*, *Juniperus phoenicea* leaves and *Ferulagummosa* roots. It exhibited antibacterial activity against several pathogenic bacteria, including *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*; antifungal activity against *Candida albicans* and *Aspergillus niger*; and cytotoxic effects against multiple human cancer cell lines, such as MCF-7, HeLaand A549 cells (Ringel *et al.*, 2022). Moreover, it has significant anti-inflammatory, antioxidant and antimicrobial activities (González *et al.*, 2012).

Similarly, β-elemeneis also a significant sesquiterpene with a molecular weight of 204.35 g/mol. It is extracted from *Curcuma rhizoma* and is employed in traditional Chinese medicine (TCM) for the treatment of various cancer types. It exhibits antitumor, anti-inflammatory and antioxidant properties (Chen *et al*., 2023; Zhai*et al*., 2019). Another widely considered aminehaving molecular weight of 155.28 g/mol is pempidine. It is well recognized for its diverse biological properties, including anticancer, analgesic, antipsychotic, antioxidant, antibacterial, antiviral and antiparasitic activities (Frolov and Vereshchagin, 2023). It is a synthetic ganglion-blocking agent with notable effects on blood pressure regulation and neuromuscular transmission. Despite its significance in hypertension treatment, it is not derived from plant sources (Corne and Edge, 1958).2-adamantanoneis also a key constituent which is basically a ketone with molecular weight of 188.28 g/mol. It exhibits significant potential for antiviral applications in the drug development industry. Additionally, it manifests neuroprotective effects and antitumor activity (Dembitsky *et al.*, 2020).

Generally, the observed variations in constituents of essential oil are likely due to fertilizer application. This finding aligns with earlier studies indicating that fertilizer use can significantly enhance phytochemical properties. Ahmadi *et al.* (2020) demonstrated that various fertilizers improved the phytochemical profile of *Echinacea purpurea*, while Jin *et al.* (2023) observed that potassium fertilizers increased antioxidant activity in lily bulbs. Besides, the agroforestry system under which the crop is being cultivated, influences the synthesis and composition of bioactive compounds. Typically, shading, tree-crop interaction, allelochemicals and soil fertility are the undeniable intercropping factors that enhance the development of enzymes and the associated biochemical processes which eventually determine the chemical profiles in secondary metabolites (Mitra *et al.*, 2022). Agroforestry systems can also create a favourable microclimate that optimizes light conditions, thereby improving the synthesis of secondary metabolites in *P. nigrum* (Oliveira *et al.*, 2018). Thus, the increased essential oil, oleoresin and phytochemical content in Bush pepper from fertilizer application and intercropping (agroforestry system) is similar to the improvements reported by Amiriyan Chelan *et al.* (2023) in *Dracocephalum moldavica* when arbuscular mycorrhizal fungi and nitrogen-fixing bacteria were used with fenugreek.

5. Conclusion

The findings of GC-MS analysis revealed a considerable shift in the concentration of phytochemical components in the essential oil of *P. nigrum*, which exhibit substantial pharmacological and biological properties, under both agroforestry system. This study further indicates that the agroforestry system has serious impact on the phytochemical composition of *P. nigrum*. This paves the way for further research into the active compounds, allowing for a deeper understanding of their pharmacological effects and mechanisms.

Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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