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## Phytochemical characterization of Mulberry (*Morus alba* L.) leaf extracts through GC-MS for exploring potential animal deterrent applications

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### Abstract

Mulberry (*Morus alba* L.) leaves contain diverse secondary metabolites with ecological and defensive functions. This study characterized the volatile and semi-volatile compounds in three mulberry accessions (MR2, V1 and S36) using GC-MS. The extracts revealed a wide range of phenolics, alkaloids, terpenoids, pyrazines and fatty acid derivatives. Key deterrent associated metabolites including 2-undecanone, caryophyllene oxide, citronellol, trans-farnesol and several pyrazines were detected, with S36 showing higher terpenoid content and MR2 exhibiting greater pyrazine abundance. The observed chemical variation among accessions provides a basis for developing mulberry-derived botanical repellents. These findings support future validation and formulation of natural, eco-friendly deterrent products.

### 1. Introduction

Secondary metabolites are low-abundance organic compounds synthesized by plants, and although they do not directly participate in basic physiological processes such as growth or respiration (Crozier *et al.*, 2006; Rehman *et al.*, 2024). They are indispensable for ecological fitness. They contribute to stress resilience, mediate plant-plant and plant-microbe interactions, and strengthen defense responses against biotic and abiotic challenges (Akula and Ravishankar, 2011; Contreras-Cornejo *et al.*, 2016; Khare *et al.*, 2020; Yang *et al.*, 2018; Jan *et al.*, 2021). Many of these compounds act as deterrents or toxins against herbivores, insects, and pathogenic organisms (Hartmann, 2004; Zaynab *et al.*, 2018; Divekar *et al.*, 2022), while others regulate signalling pathways, metabolic adjustments, and adaptive responses (Kroymann, 2011; Sana *et al.*, 2025). Plants produce a broad spectrum of secondary metabolite classes such as alkaloids, phenolics, flavonoids, terpenoids, tannins, steroids, glycosides, organic and fatty acids, volatile oils, and aromatic derivatives each associated with diverse biological or therapeutic activities (Bourgau *et al.*, 2001; Setyorini and Antarlina, 2022; Elshafie *et al.*, 2023; Gao *et al.*, 2018; Zheng *et al.*, 2021). Owing to these properties, they are widely exploited in traditional medicine, pharmaceuticals, nutraceuticals, and cosmetic formulations (Wink, 2015; Zhao *et al.*, 2005).

Mulberry (*Morus alba* L.) is a perennial, fast-growing member of the Moraceae family (Somashankar *et al.*, 2013), extensively cultivated across tropical and subtropical environments (Kumar and Chauhan, 2008). Its primary economic significance lies in its role as the exclusive food source of the silkworm *Bombyx mori*, making it fundamental to sericulture (Gautam *et al.*, 2008). Countries such as India, China, and Japan are global leaders in mulberry cultivation (Gangopadhyay, 2008), with Indian states including Tamil Nadu, Karnataka, and Andhra Pradesh contributing significantly to cocoon production (Naphade *et al.*, 2023). Beyond its agronomic value, mulberry supports agroforestry systems due to its rapid biomass production, adaptability to varied climates, soil-binding ability, and potential to generate multiple revenue sources from leaves, fruits, and other by-products (Manjunath *et al.*, 2023). Its leaves are rich in diverse secondary metabolites such as flavonoids, terpenoids, phenolic acids, alkaloids, and fatty acid derivatives linked to its medicinal, nutritional, and ecological properties (Khanal *et al.*, 2023; Zheng *et al.*, 2021).

Gas chromatography-mass spectrometry (GC-MS) remains one of the most reliable techniques for profiling volatile and semi-volatile phytochemicals due to its high sensitivity, separation efficiency and compatibility with spectral libraries like NIST and Wiley (Rubaye *et al.*, 2017). Methanol was selected as the extraction solvent because of its polarity and efficiency in recovering a broad range of plant metabolites, particularly phenolics, terpenoids, and alkaloids. GC-MS based metabolic fingerprinting is widely employed for cultivar differentiation, phytochemical quality assessment, and functional evaluation of medicinal and aromatic plants (Špánik and Machyováková, 2018). Comparative profiling also facilitates the detection of accession-specific metabolites associated with

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biochemical pathways, stress responses, or potential applications (Gao *et al.*, 2018).

Mulberry accessions exhibit substantial phytochemical diversity, and several compounds previously reported in the species including terpenoids, pyrazines, and volatile fatty acid derivatives are known to influence herbivore behavior through odor- or taste-mediated deterrence (Kumara and Yogendra, 2022). Although, the current study does not include field-based evaluations, GC-MS detection of such metabolites provides an important foundation for understanding their ecological relevance and potential applicability in natural animal-repellent formulations. The objective of the study is to characterize the volatile and semi-volatile phytochemical profiles of three mulberry (*M. alba*) accessions MR2, V1 and S36 using GC-MS and to compare their metabolite composition. This analysis enhances understanding of phytochemical variability among mulberry accessions and offers biochemical insights for their prospective use in animal deterrence and other functional applications.

## 2. Materials and Methods

### 2.1 Study area

The study was conducted in Thalavadi Block of Erode District, Tamil Nadu, India, located at an elevation of approximately 1790 m in the Dhimbham hill ranges of the Eastern Ghats along the Tamil Nadu-Karnataka interstate boundary. The region is an important sericulture zone characterized by favorable climatic conditions for mulberry cultivation. The research was carried out at the Government Large Scale Sericulture Farm, Madhahalli (785.29 acres), established in 1967 and functioning as a P1 seed farm for silkworm race improvement. This site was selected due to its extensive mulberry cultivation, availability of diverse mulberry accessions and its suitability for systematic sampling and biochemical analysis. The controlled and well-managed agricultural environment provided reliable access to uniform plant material required for GC-MS based phytochemical characterization (Figure 1).



**Figure 1: Government Large Scale Silk Farm, Madhahally, Thalavadi.**

### 2.2 Plant material collection

Fresh and healthy mulberry leaves from accessions MR2, S36 and V1 were collected from uniformly maintained 18 month old plants. For each accession, samples were collected in sterile bags, washed, shade-dried for 5-7 days, oven-dried at 45°C and ground into fine powder. The collected plant was identified from the Botanical Survey of India (BSI) No.: BSI/SRC/5/23/2025-26/Tech./825.

#### 2.2.1 MR2 variety

It is developed by Department of Sericulture, Tamil Nadu in 1970 and highly suited for high altitude areas of Tamil Nadu and also where high temperature prevails. The variety has more rooting capacity, leaf yield and growth (Figure 2).

#### 2.2.2 S36 variety

The variety is characterized by having short inter-nodes, semi-erect habit, medium branching, greyish pink coloured stem. Leaves are unlobed, cordate, glossy, and pale-green with smooth surface, with

recommended package of practices, it yields 35-45 mt/ha/year under irrigated conditions (Figure 3).



**Figure 2: MR2 variety.**



**Figure 3:** S36 variety.

### 2.2.3 V1 variety

It is a selection from controlled pollinated hybrids of S-30 and Ber, C-776 during late 1990's. The variety is characterized by erect branches and greyish stem colour. Leaves are thick, succulent, large, entire and ovate with truncate base. Leaves are smooth and glossy. Leaves are succulent and thick with very high moisture content. This variety is amendable for vegetative propagation with high rooting (90%) and good sprouting character. The leaf yield potential is more than 60 mt/ha/year (Figure 4).



**Figure 4:** V1 variety.

### 2.3 Extraction procedure

25 g of leaf powder was extracted using Soxhlet apparatus with 250 ml of analytical-grade methanol for 6 h at 60°C. The filtrate was concentrated using a rotary evaporator and stored in amber vials at 4°C (Figures 5 and 6).

### 2.4 GC-MS phytochemical screening

Dried mulberry plant material was extracted *via* Soxhlet apparatus using methanol (extracts a wide polarity range, good recovery of volatiles/semi-volatiles) at 60°C for 6 h. Cooled extracts were collected, refrigerated, and analyzed by GC-MS to detect alkaloids, flavonoids, saponins, terpenoids, tannins and phenols (Khanal *et al.*, 2023; Khan *et al.*, 2024; Rameshkumar *et al.*, 2024; Singh *et al.*, 2024). GC-MS analysis was carried out using a Perkin elmer clarus SQ8C system equipped with a DB-5MS standard non-polar capillary

column (30 m × 0.25 mm ID × 0.25 μm film thickness). A 1 μl sample of the extract was injected, and helium (He) was used as the carrier gas throughout the run. Mass spectra were acquired in electron ionization (EIz) mode, as evident in the chromatographic output and MS spectra provided in the instrument report (Figure 7).



**Figure 5:** Alkaloids extraction of powdered mulberry leaves samples using methanol.



**Figure 6:** Soxhlet extraction for GC-MS analysis.



**Figure 7:** Plant extracts obtained using Soxhlet apparatus.

### 3. Results

GC-MS analysis of the methanolic leaf extracts from the three mulberry accessions (MR2, S36 and V1) revealed a wide diversity of phytochemicals, including phenolics, alkaloids, terpenoids, fatty acids, furanones, pyrazines and other defense-related secondary metabolite classes. In total, nearly 420 compounds were detected in each accession, and from these, only metabolites with documented animal deterrent, irritant or avoidance properties were selected for detailed interpretation. Many of the shortlisted compounds have been reported in the literature for their repellent, olfactory-modifying, or deterrent effects on vertebrates and insects, underscoring their potential value in developing natural, non-lethal animal deterrent formulations.

#### 3.1 MR2 variety

The GC-MS analysis of the MR2 mulberry leaf extract revealed a broad spectrum of bioactive phytochemicals strongly associated with plant defense and potential animal deterrence. A diverse set of phenolic compounds, including 2-pyridylethanol, 5-hydroxyisovanillic acid, and methylated 1,4-benzenediol derivatives, were detected. These compounds are well known for their antimicrobial,

antioxidant, and protective functions, contributing to structural defense and reduced palatability. A substantial number of alkaloids were present, notably norbelladine, 1-deoxynojirimycin, and multiple pyrazine derivatives such as 5-butyl-2,3-dimethylpyrazine. Pyrazines are especially recognized for their intense odor and bitter sensory characteristics, which can serve as chemical warning cues or feeding deterrents to herbivores, including mammals and insects.

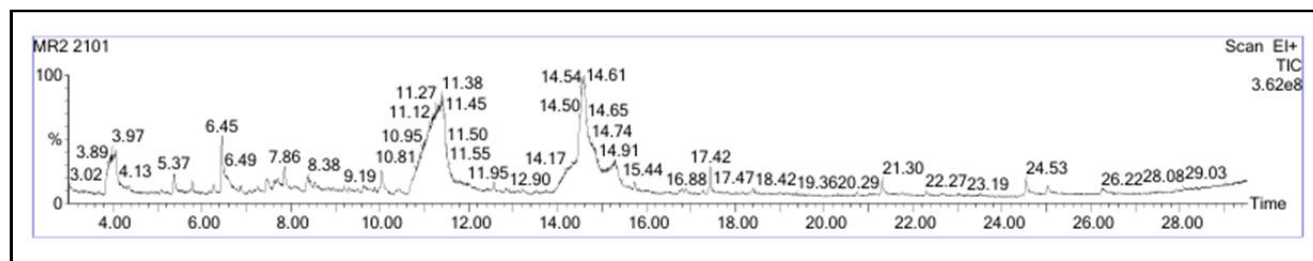
The MR2 extract also contained several terpenoids and volatile compounds notably caryophyllene oxide, R-limonene, bisabolene epoxide, and Z-8-octadecen-1-ol acetate many of which are documented for modifying animal behavior through olfactory stimulation, irritation, or repellent effects. These compounds contribute significantly to the extract's vapor-phase deterrent potential. A notable presence of fatty acid derivatives such as 2-undecanone and 2-dodecanone, both widely used natural repellents in commercial insect and vertebrate management, further highlights the strong deterrent capacity of the extract. Additionally, furanone and lactone derivatives, including tetrahydro-2H-pyranones and hydroxyketones, may add bitterness or unpalatable flavor, while sulfur and nitrogen-containing volatiles enhance the strong odor profile associated with avoidance behavior (Table 1)(Figure 8).

**Table 1: Methanolic crude extracts from MR2 variety through GC-MS profiling**

S. No.	Compound names	Retention time (in min)	Molecular formula	Molecular weight (g mol <sup>-1</sup> )	Animal deterrent
<b>Phenolic compounds</b>					
1.	2-Pyridylethanol	5.369	C <sub>7</sub> H <sub>9</sub> NO	123.15	*
2.	5-Hydroxyisovanillic acid	5.369	C <sub>8</sub> H <sub>8</sub> O <sub>5</sub>	184.15	*
3.	1,4-Benzenediol, 2-methyl-	7.710	C <sub>7</sub> H <sub>8</sub> O <sub>2</sub>	124.14	*
4.	Benzenemethanol, 3-hydroxy-	7.710	C <sub>7</sub> H <sub>8</sub> O <sub>2</sub>	124.14	*
5.	Chloramben, methyl ester	8.376	C <sub>8</sub> H <sub>7</sub> C <sub>12</sub> NO <sub>2</sub>	220.05	*
6.	2H-Pyran, 3,4-dihydro-4-hydroxy-	15.288	C <sub>5</sub> H <sub>8</sub> O <sub>2</sub>	100.12	*
7.	Bergenin	24.527	C <sub>14</sub> H <sub>16</sub> O <sub>9</sub>	328.27	*
8.	5-Methyl-2-nitrobenzyl alcohol	24.527	C <sub>8</sub> H <sub>9</sub> NO <sub>3</sub>	167.16	*
<b>Alkaloids</b>					
9.	1-Deoxynojirimycin	3.689	C <sub>6</sub> H <sub>13</sub> NO <sub>4</sub>	163.17	*
10.	Isocitronellol	7.470	C <sub>10</sub> H <sub>20</sub> O	156.269	*
11.	Norbelladine	7.710	C <sub>15</sub> H <sub>17</sub> NO <sub>3</sub>	259.30	*
12.	1-Heptanol, 2,4-dimethyl-, (2S,4R)-(-)-	11.382	C <sub>9</sub> H <sub>20</sub> O	144.25	*
13.	1-Heptanol, 2,4-dimethyl-, (R,R)-(+)-	11.382	C <sub>9</sub> H <sub>20</sub> O	144.25	*
14.	13-Heptadecyn-1-ol	11.787	C <sub>17</sub> H <sub>32</sub> O	252.44	*
15.	12-Dimethylamino-10-oxododecanoic acid	11.917	C <sub>14</sub> H <sub>27</sub> NO <sub>3</sub>	257.39	*
16.	3-Pyridinecarboxamide, N,N-diethyl-, 1-oxide	18.389	C <sub>10</sub> H <sub>14</sub> N <sub>2</sub> O <sub>2</sub>	194.23	*
17.	Pyrazine, 5-butyl-2,3-dimethyl-	18.389	C <sub>10</sub> H <sub>16</sub> N <sub>2</sub>	164.25	*
18.	2-(3-Methylbutyl)-3,5-dimethylpyrazine	18.389	C <sub>11</sub> H <sub>18</sub> N <sub>2</sub>	178.27	*
<b>Terpenoids</b>					
19.	10-Undecen-1-ol, 2-methyl-	11.917	C <sub>12</sub> H <sub>22</sub> O	182.30	*
20.	Cyclopropene, 1-methyl-3-(2-methylcyclopropyl)-	24.527	C <sub>8</sub> H <sub>12</sub>	108.18	*
21.	9-(3,3-Dimethylloxiran-2-yl)-2,7-dimethylnona-2,6-dien-1-ol	25.007	C <sub>15</sub> H <sub>26</sub> O <sub>2</sub>	238.19	*
22.	2-Hydroxy-1,1,10-trimethyl-6,9-epidioxydecalin	226.31	C <sub>13</sub> H <sub>22</sub> O <sub>3</sub>	25.007	*

Fatty acids					
23.	2-Undecanone	3.023	C <sub>11</sub> H <sub>22</sub> O	170.29	*
24.	2-Dodecanone	3.023	C <sub>12</sub> H <sub>24</sub> O	184.32	*
25.	3-Azonia-5-hexyn-1-ol, N,N-dimethyl-O-acetyl-, bromide	3.294	C <sub>9</sub> H <sub>16</sub> BrNO <sub>2</sub>	246.14	*
26.	9-Octadecenamide	8.436	C <sub>18</sub> H <sub>35</sub> NO	281.5	*
27.	10-Methyl-E-11-tridecen-1-ol propionate	11.787	C <sub>17</sub> H <sub>32</sub> O <sub>2</sub>	268.24	*
28.	Z-8-Octadecen-1-ol acetate	11.787	C <sub>20</sub> H <sub>38</sub> O <sub>2</sub>	310.51	*
29.	17-Octadecyanoic acid	11.787	C <sub>18</sub> H <sub>32</sub> O <sub>2</sub>	280.45	*
30.	10-Undecen-1-ol, 2-methyl-	11.917	C <sub>12</sub> H <sub>22</sub> O	182.30	*
31.	9,12-Octadecadienyl chloride, (Z,Z)	12.022	C <sub>18</sub> H <sub>31</sub> Cl	298.9	*
32.	Z,Z,Z-4,6,9-Nonadecatriene	12.022	C <sub>19</sub> H <sub>34</sub>	262.49	*
33.	n-Butyric acid 2-ethylhexyl ester	200.32	C <sub>12</sub> H <sub>24</sub> O <sub>2</sub>	15.288	*
34.	2-Heptenal, (Z)	15.288	C <sub>7</sub> H <sub>12</sub> O	112.17	*
35.	9,12-Octadecadienal, dimethyl acetal	15.543	C <sub>20</sub> H <sub>38</sub> O <sub>2</sub>	310.51	*
Terpenes					
36.	R-Limonene	15.543	C <sub>10</sub> H <sub>16</sub>	136.24	*
37.	Naphthalene, 1,2,3,4,4a,5,6,7-octahydro-4a-methyl	12.567	C <sub>11</sub> H <sub>18</sub>	150.26	*
38.	7-Hydroxyfarnesin	16.804	C <sub>15</sub> H <sub>24</sub> O	220.35	*
39.	4-(1-Hydroperoxy-2,2-dimethyl-6-methylene-cyclohexyl)-pent-3-en-2-one	16.804	C <sub>14</sub> H <sub>22</sub> O <sub>3</sub>	238.32	*
40.	trans-Z-α-Bisabolene epoxide	16.804	C <sub>15</sub> H <sub>24</sub> O	220.35	*
41.	trans-2-Decen-1-ol, methyl ether	21.296	C <sub>11</sub> H <sub>22</sub> O	170.29	*
42.	6-Hydroxy-7-methyl-9-oxabicyclo[3.3.1]nonan-2-one	21.296	C <sub>9</sub> H <sub>14</sub> O <sub>3</sub>	170.20	*
Furanone					
43.	2(3H)-Furanone, 5-hexyldihydro-4-methyl-, (4R-cis)-	6.450	C <sub>11</sub> H <sub>20</sub> O <sub>2</sub>	184.27	*
44.	2(3H)-Furanone, 5-hexyldihydro-4-methyl-, trans-	6.450	C <sub>11</sub> H <sub>20</sub> O <sub>2</sub>	184.27	*
45.	2(5H)-Furanone, 5-(2-methyl-3-methylene-4-butyl)-	7.470	C <sub>10</sub> H <sub>14</sub> O <sub>2</sub>	166.22	*
46.	Furan, 4-methyl-2-propyl-	7.710	C <sub>8</sub> H <sub>12</sub> O	124.18	*
47.	(±)-4-Amino-4,5-dihydro-2(3H)-furanone	7.855	C <sub>4</sub> H <sub>7</sub> NO <sub>2</sub>	101.11	*
Polyketide					
48.	2H-Pyran-2-one, tetrahydro-6-propyl	6.450	C <sub>8</sub> H <sub>14</sub> O <sub>2</sub>	142.19	*
49.	2(3H)-Furanone, dihydro-4,4-dimethyl	15.288	C <sub>6</sub> H <sub>10</sub> O <sub>2</sub>	114.14	*
50.	2,6-Naphthalenediol, 1,5-bis[[3-(4-methylpiperazino)propylimino]methyl]-	15.098	C <sub>30</sub> H <sub>46</sub> N <sub>6</sub> O <sub>2</sub>	534.75	*
Diketopiperazine					
51.	3,6-Dimethylpiperazine-2,5-dione	6.450	C <sub>6</sub> H <sub>10</sub> N <sub>2</sub> O <sub>2</sub>	142.16	*
52.	N-Ethylpiperazine-2,3-dione	6.450	C <sub>6</sub> H <sub>10</sub> N <sub>2</sub> O <sub>2</sub>	142.16	*
Pyronone					
53.	4H-Pyran-4-one, 3,5-dihydroxy-2-methyl	6.450	C <sub>6</sub> H <sub>6</sub> O <sub>4</sub>	142.11	*

Nitroimidazole derivative					
54.	1-(2-Aminoethyl)-2-methyl-5-nitroimidazole	8.376	C <sub>6</sub> H <sub>10</sub> N <sub>4</sub> O <sub>2</sub>	166.17	*
Phenylpropanoid					
55.	5-Nitro-2-toluidine	26.258	C <sub>7</sub> H <sub>8</sub> N <sub>2</sub> O <sub>2</sub>	152.15	*
56.	3-Methyl-2-nitrobenzyl alcohol	24.527	C <sub>8</sub> H <sub>9</sub> NO <sub>3</sub>	167.16	*
Pyrazine					
57.	Pyrazine, 2,5-dimethyl-3-propyl	18.389	C <sub>9</sub> H <sub>14</sub> N <sub>2</sub>	150.221	*
58.	Pyrazine, 2,5-dimethyl-3-(2-methylpropyl)	18.389	C <sub>10</sub> H <sub>16</sub> N <sub>2</sub>	164.25	*
Lipids					
59.	Oxirane, tetradecyl	25.007	C <sub>16</sub> H <sub>32</sub> O	240.42	*
Ketone					
60.	8-Hydroxy-2-octanone	11.917	C <sub>8</sub> H <sub>16</sub> O <sub>2</sub>	144.21	*
61.	2-Cyclohexen-1-one, 4-ethynyl-4-hydroxy-3,5,5-trimethyl	18.389	C <sub>11</sub> H <sub>14</sub> O <sub>2</sub>	178.23	*
62.	11-Dodecen-2-one	11.917	C <sub>12</sub> H <sub>22</sub> O	182.30	*
63.	Cyclohex-2-enone, 3-(2-dimethylaminoethylamino)-5-phenyl	11.917	C <sub>16</sub> H <sub>22</sub> N <sub>2</sub> O	258.37	*
Aldehyde					
64.	6-Nonenal, 3,7-dimethyl	10.436	C <sub>11</sub> H <sub>20</sub> O	168.27	*
65.	16-Octadecenal	266.46	C <sub>18</sub> H <sub>34</sub> O	11.787	*
Steroid					
66.	Allopregnane-7 $\alpha$ ,11 $\alpha$ -diol-3,20-dione	15.543	C <sub>21</sub> H <sub>32</sub> O <sub>4</sub>	348.48	*
Amino acid					
67.	2-Acetylamino-3-mercapto-3-methylbutyric acid	15.728	C <sub>7</sub> H <sub>13</sub> NO <sub>3</sub> S	191.25	*
Thioether					
68.	Ethanethiol, 2-(4-(p-methoxyphenyl)butyl)amino-1-methyl-, hydrogen thiosulfate	15.728	C <sub>15</sub> H <sub>25</sub> NO <sub>3</sub> S <sub>2</sub>	327.5	*
Thiole					
69.	2,7,11-Trimethyl-4-phenylthiododeca-2,6,10-triene	16.804	C <sub>21</sub> H <sub>30</sub> S	314.5	*
Verbenone					
70.	Bicyclo[3.1.1]hept-3-en-2-one, 4,6,6-trimethyl	12.567	C <sub>10</sub> H <sub>14</sub> O	150.22	*
Other categories					
71.	2-Penten-1-ol, 2-methyl	4.354	C <sub>6</sub> H <sub>12</sub> O	100.16	*
72.	Taurine	5.369	C <sub>2</sub> H <sub>7</sub> NO <sub>3</sub> S	125.15	*



**Figure 8: Methanolic crude extracts from MR2 variety through GC-MS profiling.**

### 3.2 S36 variety

The GC-MS analysis of the S36 mulberry leaf extract revealed a diverse array of phytochemicals spanning phenolics, alkaloids, terpenoids, fatty acids, ketones, and additional defense-related

metabolites, many of which are associated with plant protective functions and animal deterrence. Prominent phenolic compounds such as 3-cyano-7-hydroxycoumarin and caffeic acid were detected, both known for their antioxidant, antimicrobial, and herbivore-

deterrent roles in plants. The presence of hydroquinine, another phenolic derivative, further supports the extract's defensive biochemical profile. A rich composition of alkaloids was observed, including 1- and 5-isoquinolinecarbonitrile, pyridine derivatives (2-phenyl and 3-phenyl pyridine), and hydroxychloroquine. These compounds are commonly associated with bitterness, neuroactivity, or mild toxicity, contributing to unpalatability and potential feeding deterrence in herbivores. The extract also contained multiple terpenoids and terpenes, such as trans-farnesol, R-limonene, cyclohexanol derivatives, and 1, 6-octadiene derivatives, all of which are recognized for their volatile odor profiles and well-documented roles in insect and vertebrate repellency.

Several fatty acid esters and ketones were identified, including 3-decanoyic acid, methylbutenoate esters (tridecyl, octyl, and decyl), 2-undecanone, and 2-tridecanone. These compounds are widely reported in natural repellent studies due to their irritant properties and ability to influence olfactory-driven avoidance behavior in animals. Additional metabolites such as pyranones, lactones, nitriles, quinoline derivatives, and acetic acid esters contribute to the chemical complexity of the extract, further enhancing its potential deterrent efficacy. Overall, the phytochemical composition of the S36 variety highlights its strong potential as a natural, non-lethal animal repellent, supported by the presence of multiple bioactive compounds across diverse chemical groups (Table 2) (Figure 9).

**Table 2: Methanolic crude extracts from S36 variety through GC-MS profiling**

S. No.	Compound names	Retention time (in min)	Molecular formula	Molecular weight (g mol <sup>-1</sup> )	Animal repellent
<b>Phenolic compounds</b>					
1.	3-Cyano-7-hydroxycoumarin	5.009	C <sub>13</sub> H <sub>12</sub> O	198.34	*
<b>Alkaloids</b>					
2.	1-Isoquinolinecarbonitrile	13.483	C <sub>10</sub> H <sub>6</sub> N <sub>2</sub>	154.17	*
3.	5-Isoquinolinecarbonitrile	13.483	C <sub>10</sub> H <sub>6</sub> N <sub>2</sub>	154.17	*
4.	Pyridine, 2-phenyl-	13.483	C <sub>11</sub> H <sub>9</sub> N	155.20	*
5.	Pyridine, 3-phenyl	13.483	C <sub>11</sub> H <sub>9</sub> N	155.20	*
6.	Benzoic acid, 4-[(chloroamino)sulfonyl]	14.083	C <sub>7</sub> H <sub>6</sub> ClNO <sub>4</sub> S	235.64	*
7.	6-Methyl-5-octen-2-one	14.083	C <sub>9</sub> H <sub>16</sub> O	140.22	*
8.	2-Hexen-1-ol, acetate, (E)-	14.083	C <sub>8</sub> H <sub>14</sub> O <sub>2</sub>	142.19	
9.	3-Hexen-1-ol, (E)	14.203	C <sub>6</sub> H <sub>12</sub> O	100.16	*
10.	Hydroxychloroquine	14.613	C <sub>18</sub> H <sub>26</sub> ClN <sub>3</sub> O	335.88	*
<b>Terpenoids</b>					
11.	Cyclohexanol, 4-methyl-	3.399	C <sub>7</sub> H <sub>14</sub> O	114.19	*
12.	1,6-Octadiene, 8-methoxy-	11.042	C <sub>9</sub> H <sub>16</sub> O	140.226	*
13.	8-Hydroxy-2,6-dimethylocta-2,6-dienoic acid, ethyl ester	11.332	C <sub>12</sub> H <sub>20</sub> O <sub>3</sub>	212.28	*
<b>Fatty acids</b>					
14.	3-Decanoyic acid	11.332	C <sub>11</sub> H <sub>22</sub> O <sub>2</sub>	186.29	*
15.	3-Methyl-2-butenoic acid, tridecyl ester	14.083	C <sub>18</sub> H <sub>32</sub> O <sub>2</sub>	280.45	*
16.	3-Methyl-2-butenoic acid, octyl ester	14.083	C <sub>13</sub> H <sub>24</sub> O <sub>2</sub>	212.328	*
17.	3-Methyl-2-butenoic acid, decyl ester	14.083	C <sub>15</sub> H <sub>28</sub> O <sub>2</sub>	240.38	*
18.	Z-(13,14-Epoxy)tetradec-11-en-1-ol acetate	14.203	C <sub>16</sub> H <sub>28</sub> O <sub>3</sub>	268.397	*
<b>Terpenes</b>					
19.	(Z)-(E)-2-Methylbut-2-en-1-yl 2-methylbut-2-enoate	5.780	C <sub>10</sub> H <sub>16</sub> O <sub>2</sub>	168.23	*
20.	trans-Farnesol	11.527	C <sub>15</sub> H <sub>26</sub> O	222.37	*
21.	R-Limonene	14.203	C <sub>10</sub> H <sub>16</sub>	136.23	*
<b>Phenylpropanoid</b>					
22.	Caffeic acid	5.009	C <sub>9</sub> H <sub>8</sub> O <sub>4</sub>	180.16	*

Ketone					
23.	2-Undecanone, 6,10-dimethyl-dialkylketone	11.777	C <sub>13</sub> H <sub>26</sub> O	198.34	*
24.	2-Tridecanone	11.777	C <sub>13</sub> H <sub>26</sub> O	198.34	*
Other categories					
25.	Pentanedinitrile, 2-methyl	6.275	C <sub>6</sub> H <sub>8</sub> N <sub>2</sub>	108.14	*
26.	2H-Pyran-2-one, tetrahydro-6-propyl	6.450	C <sub>8</sub> H <sub>14</sub> O <sub>2</sub>	142.19	*
27.	2-Heptadecanone	11.777	C <sub>13</sub> H <sub>15</sub> O	254.46	*
28.	4-Hydroxy-2-methylpyrrolidine-2-carboxylic acid	14.083	C <sub>6</sub> H <sub>11</sub> NO <sub>3</sub>	145.16	*
29.	Acetic acid, 2-(5,9-dimethyl-1-oxacycloundeca-5,9-dien-2-ylidene)	14.203	C <sub>15</sub> H <sub>22</sub> O <sub>3</sub>	250.34	*
30.	Formic acid, 2-(2-methoxyethyl)hexyl ester	14.413	C <sub>10</sub> H <sub>20</sub> O <sub>3</sub>	188.2640	*
31.	Hydroquinine	14.613	C <sub>6</sub> H <sub>6</sub> O <sub>2</sub>	110.11	*

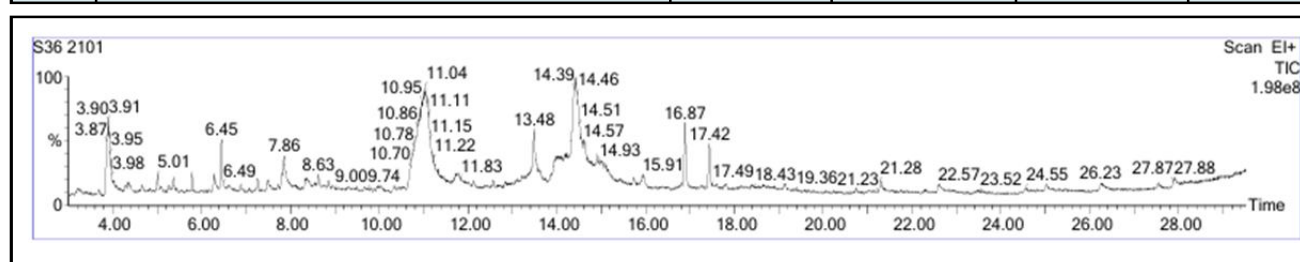


Figure 9: Methanolic crude extracts from S36 variety through GC-MS profilin g.

### 3.3 V1 variety

The GC-MS analysis of the V1 mulberry leaf extract revealed a diverse but comparatively moderate profile of phytochemicals spanning phenolics, alkaloids, terpenoids, fatty acids, terpenes, and other defense-related metabolites. Among phenolic constituents, compounds such as *m*-guaiacol and 2-(dimethylamino)-phenol were identified; both are known for their antimicrobial and antioxidant activities, which can enhance the plant's defensive capacity. Notable alkaloids, including 1, 4-pentanediamine and tetrahydropyridine derivatives, were detected. These compounds are commonly associated with neuroactive or bitter sensory properties that contribute to herbivore deterrence through unpalatability or mild toxicity.

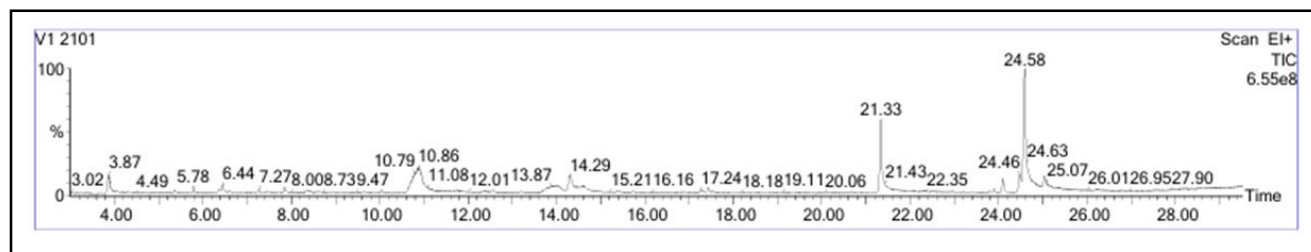
The V1 extract also exhibited a range of terpenoids and terpenes, including  $\alpha$ -citronellol, 1,4-dihydroxy-*p*-menth-2-ene, *D*-verbenone, and *trans-Z*- $\alpha$ -bisabolene epoxide. These volatiles are well documented for their strong aromatic profiles and insect-repellent functions,

forming a key component of plant chemical defense. Additional terpenoid-related compounds such as hexahydronaphthalenone and trimethylbicycloheptanone further strengthen the volatile-mediated deterrent properties of the extract. Several fatty acid derivatives—namely methyl 9-heptadecenoate, 3-nonenic acid ethyl ester, and 4-decenoic acid (3-methyl-, *E*)-were present. These compounds are recognized for their roles in herbivore deterrence through odor-mediated avoidance, irritant activity, or involvement in plant signaling pathways. Beyond these major classes, the extract contained additional defensive metabolites such as the alkene *Z,Z,Z*-1,4,6,9-nonadecatetraene, the phenylpropanoid 2-pyridinecarboxylic acid, 5-nitro-, the benzene derivative acetic acid, phenyl ester, and the polyketide 2,4(3H,5H)-furanone, 5-methyl-3-phenyl-. Ketones such as 7-octen-2-one and 6-octen-2-one (*Z*)-known components of insect semiochemical systems were also identified. Long-chain hydrocarbons like 11-heneicosanone and esters including formic acid, 2-(2-methoxyethyl) hexyl ester further enhance the chemical complexity and potential repellency of the extract (Table 3) (Figure 10).

Table 3: Methanolic crude extracts from V1 variety through GC-MS profiling

S. No.	Compound names	Retention time (in min)	Molecular formula	Molecular weight (g mol <sup>-1</sup> )	Animal repellent
Alkaloids					
1.	1,4-Pentanediamine	3.033	C <sub>18</sub> H <sub>27</sub> Cl <sub>2</sub> N <sub>3</sub>	356.3	*
2.	Pyridine, 1,2,3,6-tetrahydro-1,2-dimethyl-	3.359	C <sub>7</sub> H <sub>13</sub> N	111.18	*
Terpenoids					
3.	$\alpha$ -Citronellol	5.785	C <sub>10</sub> H <sub>20</sub> O	156.269	*
4.	5-Nonanol, 5-methyl-	7.845	C <sub>10</sub> H <sub>22</sub> O	158.28	*

5.	Cyclopropane, (methoxymethyl)-	11.762	C <sub>5</sub> H <sub>10</sub> O	86.13	*
6.	(3S,4aR,5S,8aS)-4a,5-Dimethyl-3-(prop-1-en-2-yl)-2,3,4,4a,5,6 hexahydronaphthalen-1(8aH)-one	12.567	C <sub>15</sub> H <sub>22</sub> O	218.33	*
7.	4,7,7-Trimethylbicyclo[4.1.0]hept-3-en-2-one	12.567	C <sub>16</sub> H <sub>24</sub> O	150.22	*
<b>Fatty acids</b>					
8.	Methyl 9-heptadecenoate or 9-17:1	3.304	C <sub>18</sub> H <sub>34</sub> O <sub>2</sub>	282.46	*
9.	3-Nonenoic acid, ethyl ester	3.359	C <sub>11</sub> H <sub>20</sub> O <sub>2</sub>	184.27	*
10.	4-Decenoic acid, 3-methyl-, (E)-	5.359	C <sub>12</sub> H <sub>22</sub> O <sub>3</sub>	184.27	*
11.	2-Pentenoic acid	13.998	C <sub>5</sub> H <sub>8</sub> O <sub>2</sub>	100.12	*
<b>Terpenes</b>					
12.	10-Undecen-1-al, 2-methyl-	14.303	C <sub>12</sub> H <sub>22</sub> O	182.30	*
13.	trans-Z- $\alpha$ -Bisabolene epoxide	12.567	C <sub>14</sub> H <sub>24</sub> O	220.35	*
<b>Benzene</b>					
14.	Acetic acid, phenyl ester	6.600	C <sub>8</sub> H <sub>8</sub> O	136.15	*
<b>Ketone</b>					
15.	7-Octen-2-one	14.303	C <sub>8</sub> H <sub>14</sub> O	126.19	*
<b>Formic acid</b>					
16.	Formic acid, 2-(2-methoxyethyl)hexyl ester	14.303	C <sub>10</sub> H <sub>20</sub> O <sub>3</sub>	188.26	*
<b>Hydrocarbon</b>					
17.	11-Heneicosanone	17.259	C <sub>21</sub> H <sub>44</sub>	296.58	*



**Figure 10: Methanolic crude extracts from V1 variety through GC-MS profiling.**

#### 4. Discussion

The GC-MS based phytochemical characterization of mulberry (*M. alba*) leaf extracts revealed a chemically diverse profile rich in phenolics, alkaloids, terpenoids, pyrazines and fatty acid derivatives, many of which are known to play critical roles in plant defense and herbivore deterrence. Compounds such as caryophyllene oxide,  $\alpha$ -citronellol, trans-farnesol, R-limonene, 1-deoxynojirimycin, and 2-undecanone possess documented odor-mediated repellency, irritant properties or bitter taste profiles that can influence the foraging behavior of mammals (Estell *et al.*, 2008; Culebras *et al.*, 2017). In comparison, the S36 accession showed the highest diversity of terpenoids, which are commonly linked to strong odor based repellency, whereas MR2 exhibited greater abundance of pyrazines and ketone derivatives associated with irritant and avoidance responses in mammals. The V1 accession displayed a comparatively moderate profile with fewer deterrent-related volatiles. This variation highlights the differing deterrent potentials among the three mulberry

accessions. The presence of these bioactive metabolites across the MR2, S36, and V1 varieties underscores the inherent defensive chemistry of mulberry leaves and suggests strong potential for their application as natural deterrents (Kumara and Yogendra, 2022). These findings align with growing interest in plant-derived repellents as sustainable, eco-friendly alternatives to synthetic chemicals, highlighting mulberry extracts as promising candidates for developing non-lethal strategies to mitigate human-wildlife conflict in agricultural landscapes.

#### 5. Conclusion

The GC-MS based phytochemical characterization of mulberry (*M. alba*) leaf extracts from the MR2, S36 and V1 accessions revealed a rich diversity of volatile and semi-volatile metabolites, including alkaloids, phenolics, terpenoids, pyrazines, fatty acid derivatives and other bioactive compounds with known ecological roles in plant defense and animal deterrence. Notably, S36 exhibited the highest

number of terpenoids, while MR2 showed a greater abundance of pyrazines and ketone derivatives, indicating its stronger volatile-mediated deterrent potential. The consistent presence of deterrent-associated compounds such as 1-deoxynojirimycin, caryophyllene oxide,  $\alpha$ -citronellol, trans-farnesol, R-limonene and 2-undecanone underscores the inherent chemical defense capacity of mulberry leaves. These findings provide a strong biochemical basis for developing mulberry-derived botanical repellents as sustainable and eco-friendly solutions for mitigating wildlife-induced crop damage. Future research should focus on bioassay-guided fractionation, isolation and characterization of the most active deterrent compounds, and field-level efficacy testing against target animals under varied agro-ecological conditions. Additionally, emphasis should be placed on formulation optimization and product development, enabling scalable, farmer-ready repellent solutions suitable for forest-fringe agricultural landscapes.

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### Conflict of interest

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

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