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Research paper

## Comparative GC-MS analysis of *Withania somnifera* (L.) Dunal grown at high temperature

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### ABSTRACT

**Context:** Ashwagandha (*Withania somnifera*) (L.) Dunal is a medicinal plant from family *Solanaceae* valued for its diverse secondary metabolites, which contribute to its therapeutic importance. **Objective:** This study examined how elevated temperature influences the phytochemical profile of Ashwagandha roots. **Methods:** Plants were cultivated under two controlled conditions: an ambient temperature (27–32 °C) and an elevated temperature at poly-tunnel to mimic global warming (35–36 °C). GC–MS analysis was conducted. **Results:** Both temperature treatments exhibited comparable molecular weight distributions and shared several recurring molecular signatures, indicating conservation of core metabolic pathways. However, the ambient-grown sample (27°C –32 °C) displayed pronounced metabolite dominance, with three major peaks accounting for nearly 65% of total chromatographic area and a broader molecular weight range. In contrast, plants exposed to elevated temperature (35°C – 36 °C) showed reduced peak dominance, lower relative abundance of lipid-associated masses, and a more even distribution of metabolite signals, suggesting redistribution of metabolic flux under temperature stress. **Conclusion:** These findings indicate that while temperature stress does not eliminate core metabolic activity in *W. somnifera* roots, it significantly alters quantitative metabolite distribution. Ambient conditions appear to favor greater metabolite concentration and structural stability, whereas elevated temperature promotes metabolic rebalancing that may influence phytochemical consistency and medicinal quality.

**Keywords:** Temperature stress, global warming

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## INTRODUCTION

In Sri Lanka, *W. somnifera* (L.) Dunal (Solanaceae) is commonly known as Amukkara (Sinhala: අමුක්කරා) and Amukkira/Amukkiray (Tamil), and it is incorporated into Ayurvedic and indigenous medical systems primarily as a restorative tonic aimed at enhancing physical strength, vitality, and stress resilience (Paul et al., 2021). Traditionally, the root is the principal medicinal part and is included in formulations prescribed for general debility, nervous disorders, inflammatory conditions, and stress-related ailments (Singh et al., 2011). Because dosage recommendations vary depending on preparation type (powdered root vs. standardized extract) and practitioner guidance, evidence-based ranges from human clinical studies are reported for context. Clinical investigations and authoritative health monographs indicate that commonly studied doses range from 240–1,250 mg/day of standardized root extract, or up to approximately 6 g/day of dried root powder equivalent, depending on formulation and therapeutic indication (National Institutes of Health [NIH], 2023; Singh et al., 2011)

In Sri Lankan traditional medicine, *W. somnifera* is employed in the management of inflammatory conditions, emaciation, dermatological and gastrointestinal disorders, insomnia, respiratory ailments, neurological disturbances, reproductive dysfunction, and general weakness, and is widely prescribed as a restorative tonic within Ayurvedic practice (Paul et al., 2021; Singh et al., 2011). Contemporary pharmacological investigations support many of these traditional applications, reporting anxiolytic, antioxidant, anti-inflammatory, immunomodulatory, neuroprotective, hepatoprotective, and adaptogenic activities, with demonstrated effects on the nervous, endocrine, and reproductive systems (Mirjalili et al., 2009; Paul et al., 2021).

The principal bioactive constituents of *W. somnifera* are withanolides, a group of steroidal lactones that include withaferin A, withanolide A, and withanolide D. These compounds have been associated with anti-inflammatory, immunomodulatory, neuroprotective, and anticancer-related activities in experimental studies (Mirjalili et al., 2009; Paul et al., 2021). Regarding safety, authoritative evaluations indicate that ashwagandha is generally well tolerated when used short term at recommended doses; however, mild adverse effects such as gastrointestinal discomfort have been reported. Potential interactions with sedatives, immunosuppressants, or thyroid medications should be considered, and rare cases of liver injury have been documented (National Institutes of Health [NIH], 2023).

Understanding how elevated temperatures influence Ashwagandha's root phytochemical dynamics and antioxidant responses is increasingly important under climate change, as prolonged periods of thermal stress can significantly alter the plant's chemistry and therapeutic potential (Jha et al., 2025; A. Singh & Mina, 2025). Changes in temperature may reduce the concentration of crucial bioactive compounds or shift metabolic pathways in ways that influence the plant's therapeutic value. Gas Chromatography Mass Spectrometry (GC-MS) provides a powerful platform for profiling volatile and semi-volatile metabolites and assessing how environmental factors reshape chemical diversity in roots.

This study investigates the impact of elevated temperature on the secondary-metabolite profile of *W. somnifera* roots grown under controlled conditions. By comparing GC-MS profiles of plants cultivated at ambient and elevated temperatures, we aim to determine whether thermal stress alters metabolite classes, abundance, or chemical complexity. Such insights are essential for ensuring cultivation practices that preserve the phytochemical integrity and medicinal quality of Ashwagandha.

## MATERIALS AND METHODS

### *Plant material*

Pest, disease free mother plants of Ashwagandha were selected and vegetatively propagated in a nursery to generate uniform plant material for the experiment. Propagules were maintained for two and a half months before being transferred to experimental conditions. The nursery substrate consisted of a mixture of reddish-brown earth (RBE) soil, coir dust, and compost at a ratio of 3:1:1. Pots measuring 20 cm in diameter and 25 cm in height were irrigated until saturation to ensure adequate soil water capacity during plant establishment. For the temperature treatments, plants were grown either under ambient conditions (27°C -32 °C) in a naturally ventilated plant house or under elevated temperature conditions (35°C -36 °C) inside a temperature regulated poly tunnel. These temperatures selected based on IPCC result on global climate (IPCC, 2007) and the HadCM3 predictions of Sri Lankan air temperature in 2050 for A2 scenario of IPCC (Silva et al., 2007). Temperatures in the temperature-regulated polytunnel and plant house were monitored using installed mercury glass thermometers positioned at canopy height. Readings were recorded three times daily (6:00–8:00 a.m., 12:00–2:00 p.m., and 4:00–6:00 p.m.) throughout the experimental period. In the polytunnel, a thermostat-controlled ventilation system was automatically activated when temperatures exceeded the preset threshold to maintain the desired range. All plants which were taken to this experiment were grown in the same soil–compost mixture containing 1 kg of compost and 9 kg of soil to standardize nutrient availability (Filipović et al., 2023; Gupta et al., 2016). Irrigation was applied uniformly across treatments to maintain adequate soil moisture (watered daily) and plants were inspected regularly to avoid water stress.



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Figure 1: *W. somnifera* plants grown under ambient temperature conditions (27°C –32 °C) with compost treatment. Representative samples (T1M1R1, T1M1R2, and T1M1R3) illustrate uniform vegetative growth prior to root harvest for GC–MS analysis.

### ***Extraction***

The roots of Ashwagandha were chosen for this study as they are the most extensively used medicinal component in Ayurvedic practice and modern herbal formulations, containing numerous active biochemicals associated with tonic, aphrodisiac, anxiolytic, sedative, antioxidant, and immunomodulatory properties, with efficacy demonstrated in both experimental and clinical studies (Kumar et al., 2022; Langade et al., 2019; Mikulska et al., 2023). Roots were harvested, washed, and dried under shade at room temperature to minimize photodegradation of metabolites. The dried roots were ground into a fine powder and extracted using Methanol ( $\geq 99.8\%$  GC/HPLC grade; Sigma-Aldrich, St. Louis, MO, USA) at a 1:10 (w/v) ratio. Extraction was carried out in conical flasks sealed with aluminum foil to prevent solvent evaporation and incubated on an orbital shaker (50 rpm) for 48 hours at room temperature. After incubation, the mixture was filtered through Whatman No. 1 filter paper, and the resulting filtrate was concentrated by evaporating methanol in an incubator at 37 °C. The dried crude extract was subsequently stored at 4 °C until further analysis, following procedures similar to those described by (Ingkaninan et al., 2003).

### ***Gas Chromatography-Mass Spectrometry (GC-MS)***

GC-MS profiling was conducted using a Shimadzu GCMS-QP2010 Plus system equipped with an H5 capillary column. The oven temperature program began at 80 °C with a 2-minute hold, followed by a ramp to 250 °C at 5 °C/min. A 1.0  $\mu$ L aliquot of the methanolic extract was injected in split mode with a 10:1 split ratio. Helium served as the carrier gas at a constant flow rate of 1.2 mL/min. The injector and mass transfer line temperatures were maintained at 250 °C. Ionization was carried out by electron impact (EI) at 70 eV, and mass spectra were recorded over an m/z range of 40-600 during a 50-minute run. Compound identification was performed by comparing the resulting spectra with entries in the NIST 14 Mass Spec Library.

## **RESULTS**

### ***GC-MS analysis of sample grown at ambient temperature (T1; 27–32 °C)***

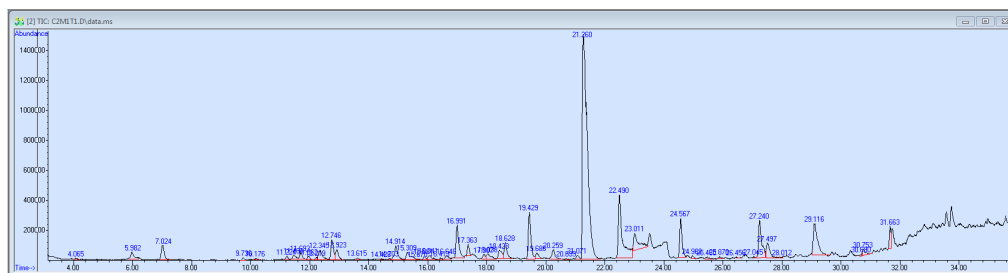
GC-MS analysis of *W.somnifera* root extracts grown under ambient temperature revealed 37 detectable compounds representing a mixture of primary metabolic products and putative specialized metabolites (Table 1; Figure 1). The chromatographic profile was strongly dominated by three peaks: Retention Time (RT) 31.669 min (Area 34.85%; Molecular Weight (MW) 299.32;  $C_{12}H_{24}O_2$ ), RT 21.34 min (Area 25.05%; MW 127.18;  $C_7H_{13}NO$ ), and RT 27.017 min (Area 5.61%; MW 256.43;  $C_{16}H_{32}O_2$ ). Together, these accounted for approximately 65% of total chromatographic area, indicating substantial metabolite dominance under non-stress conditions.

Based on molecular formula patterns, the most abundant compounds are consistent with medium-chain lipid derivatives ( $C_{12}H_{24}O_2$ ;  $C_{16}H_{32}O_2$ ), typically associated with fatty acid metabolism and membrane lipid turnover (Ruelland & Zachowski, 2010).

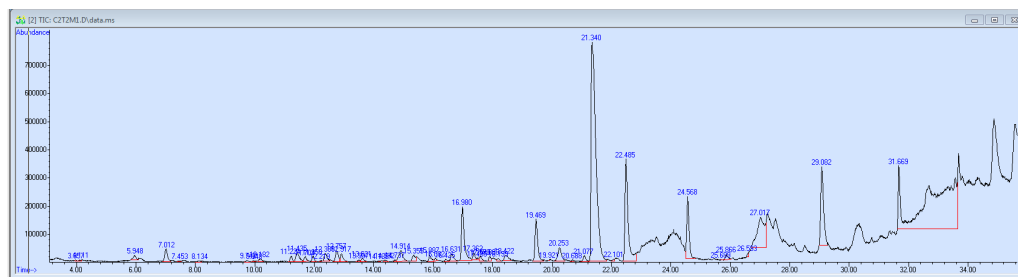
The second most abundant compound ( $C_7H_{13}NO$ ) represents a nitrogen-containing molecule potentially derived from amino acid or alkaloid-associated pathways. Additional moderately abundant peaks included RT 16.98 min (Area 3.64%; MW 154.25;  $C_{10}H_{18}O$ ) and RT 24.568 min (Area 3.47%; MW 72.06;  $C_3H_4O_2$ ), corresponding to oxygenated hydrocarbons and small organic acids, which reflect active primary carbon metabolism. The broad molecular weight distribution (56–424 g) suggests substantial chemical richness under ambient growth conditions.

#### ***GC-MS analysis of sample grown at temperature stress (T2; 35°C –36 °C)***

Roots exposed to elevated temperature exhibited 34 detectable compounds, indicating a slight reduction in chemical richness compared to ambient conditions. The three highest peaks were observed at RT 19.429 min (Area 4.49%; MW 127.18;  $C_7H_{13}NO$ ), RT 27.24 min (Area 4.43%; MW 223.31;  $C_{13}H_{21}NO_2$ ), and RT 16.991 min (Area 3.46%; MW 154.25;  $C_{10}H_{18}O$ ). Notably, no individual compound exceeded 5% of total area, indicating loss of metabolite dominance under temperature stress. The persistence of  $C_7H_{13}NO$  across treatments suggests relative stability of nitrogen-associated metabolism. However, lipid-associated compounds that dominated under ambient conditions were less abundant under elevated temperature, suggesting temperature-driven shifts in carbon allocation or membrane lipid transformation. Under temperature stress samples also showed the presence of higher molecular weight derivatives (296 g –503 g), including silicon-containing compounds, likely reflecting derivatization products or stress-induced metabolic turnover. Overall, elevated temperature redistributed metabolite abundance rather than eliminating major molecular classes. Several molecular masses were detected in both treatments, including MW 154.25 ( $C_{10}H_{18}O$ ), 85.15 ( $C_5H_{11}N$ ), and 128.17 ( $C_{10}H_8$ ). These shared signs indicate conservation of core metabolic pathways despite of temperature variation. However, relative abundance differences demonstrate quantitative change of metabolic flux under temperature stress. Because compound identities were not confirmed with authenticated standards, all metabolite classifications remain tentative and are based on molecular mass and formula.



**Figure 2.** Chromatogram of Ashwagandha (*W. somnifera*) root extract grown under ambient temperature conditions (27-32 °C).



**Figure 3.** Chromatogram of Ashwagandha (*W. somnifera*) root extract grown under elevated temperature stress (35-36 °C).

## DISCUSSION

This study demonstrates that temperature stress (35°C –36 °C) alters the quantitative distribution of metabolites in *W. somnifera* roots while maintaining core metabolic pathways. Under ambient conditions, three dominant metabolites accounted for approximately 65% of total chromatographic area, suggesting preferential metabolic channeling into lipid-associated and nitrogen-containing molecular classes. In contrast, elevated temperature reduced peak dominance and redistributed metabolite abundance across a broader spectrum of compounds. Temperature stress is known to disrupt membrane stability, modify lipid composition, and alter metabolic flux patterns as part of plant acclimation responses (Bita & Gerats, 2013; Ruelland & Zachowski, 2010). The reduction in dominance of lipid-associated compounds observed under elevated temperature treatment (35°C –36 °C) might reflect temperature-induced membrane remodeling and carbon reallocation processes. The persistence of nitrogen-containing molecular signs across both treatments indicates stability of amino acid associated or alkaloid-related metabolic routes. Environmental factors, including temperature fluctuations, are well established modulators of specialized metabolism in medicinal plants (Yang et al., 2018). The redistribution of metabolite abundance observed in this study aligns with reports that environmental stress reshapes phytochemical balance without necessarily suppressing entire biosynthetic pathways. Importantly, GC–MS primarily captures volatile and semi-volatile constituents (Attanayake et al, 2025). Major pharmacologically active compounds of *W. somnifera*, including withanolides (steroidal lactones), are more accurately quantified using LC–MS or HPLC-based analytical approaches (Mirjalili et al., 2009). Thus, while the present findings demonstrate temperature-associated modulation of volatile metabolite architecture, further targeted analysis is necessary to determine whether key bioactive steroidal compounds are similarly affected. Overall, temperature stress appears to induce metabolic reorganization rather than pathway loss, reflecting biochemical plasticity that may support environmental adaptation.

## CONCLUSION

Temperature significantly influences the volatile metabolite composition of *W. somnifera* roots. Ambient conditions promoted strong metabolite dominance, with three compounds accounting for nearly two-thirds of total abundance. Elevated

temperature reduced dominance and redistributed metabolite abundance across a broader range of compounds. Despite quantitative shifts, major molecular classes including lipid-associated compounds, nitrogen-containing molecules, and oxygenated hydrocarbons were conserved under both temperature regimes. These findings indicate that temperature stress induces metabolic rebalancing while maintaining core biosynthetic capacity. From a public health perspective, these results are particularly relevant to Sri Lanka, where *W. somnifera* is widely incorporated into Ayurvedic formulations and traditional medicinal systems.

As climate change contributes to rising temperatures and increased thermal variability in tropical regions, understanding how heat stress affects phytochemical composition is essential for maintaining the quality, safety, and therapeutic consistency of plant-derived medicinal products. Future research should quantify withanolides under controlled temperature gradients, integrate transcriptomic and metabolomic profiling to identify heat-responsive regulatory pathways, and assess whether temperature-associated chemical shifts influence pharmacological efficacy.

**Table 1.** GC-MS analysis of *W. somnifera* grown at ambient temperature (27-32 °C)

No	RT (min)	Area %	Molecular mass (g)	Molecular Formula
1	3.957	0.03	177.16	C <sub>6</sub> H <sub>11</sub> NO <sub>4</sub>
2	4.111	0.06	62.03	H <sub>2</sub> CO <sub>3</sub>
3	7.012	0.81	88.11	C <sub>4</sub> H <sub>8</sub> O <sub>2</sub>
4	7.453	0.05	94.11	C <sub>5</sub> H <sub>6</sub> N <sub>2</sub>
5	8.134	0.1	123.20	C <sub>8</sub> H <sub>13</sub> N
6	9.948	0.08	424.79	C <sub>29</sub> H <sub>60</sub> O
7	10.182	0.17	70.09	C <sub>4</sub> H <sub>6</sub> O
8	11.224	0.31	228.42	C <sub>15</sub> H <sub>32</sub> O
9	11.435	0.74	56.06	C <sub>3</sub> H <sub>4</sub> O
10	12.219	0.04	135.13	C <sub>5</sub> H <sub>5</sub> N <sub>5</sub>
11	12.368	0.5	101.15	C <sub>5</sub> H <sub>11</sub> NO
12	12.757	0.74	152.23	C <sub>10</sub> H <sub>16</sub> O
13	12.917	0.53	218.21	C <sub>11</sub> H <sub>10</sub> N <sub>2</sub> O <sub>3</sub>
14	13.507	0.11	102.13	C <sub>5</sub> H <sub>10</sub> O <sub>2</sub>
15	14.188	0.03	254.41	C <sub>16</sub> H <sub>30</sub> O <sub>2</sub>
16	14.382	0.11	59.11	C <sub>3</sub> H <sub>9</sub> N
17	14.914	1.04	84.16	C <sub>6</sub> H <sub>12</sub>
18	15.355	0.42	137.14	C <sub>7</sub> H <sub>7</sub> NO <sub>2</sub>
19	15.887	0.37	238.46	C <sub>17</sub> H <sub>34</sub>
20	16.082	0.13	98.10	C <sub>5</sub> H <sub>6</sub> O <sub>2</sub>
21	16.425	0.12	96.17	C <sub>7</sub> H <sub>12</sub>
22	16.631	0.52	85.10	C <sub>4</sub> H <sub>7</sub> NO

23	16.98	3.64	154.25	C <sub>10</sub> H <sub>18</sub> O
24	17.363	0.33	80.13	C <sub>6</sub> H <sub>8</sub>
25	17.598	0.26	128.17	C <sub>10</sub> H <sub>8</sub>
26	18.199	0.13	112.09	C <sub>4</sub> H <sub>4</sub> N <sub>2</sub> O <sub>2</sub>
27	18.422	0.51	204.36	C <sub>15</sub> H <sub>24</sub>
28	19.469	2.66	123.11	C <sub>6</sub> H <sub>5</sub> NO <sub>2</sub>
29	19.921	0.02	125.14	C <sub>5</sub> H <sub>7</sub> N <sub>3</sub>
30	20.253	1.19	100.16	C <sub>6</sub> H <sub>12</sub> O
31	21.077	0.37	112.17	C <sub>7</sub> H <sub>12</sub> O
32	21.34	<b>25.05</b>	127.18	C <sub>7</sub> H <sub>13</sub> NO
33	22.101	0.07	100.12	C <sub>5</sub> H <sub>8</sub> O <sub>2</sub>
34	24.568	3.47	72.06	C <sub>3</sub> H <sub>4</sub> O <sub>2</sub>
35	26.593	0.11	214.35	C <sub>13</sub> H <sub>26</sub> O <sub>2</sub>
36	27.017	5.61	256.43	C <sub>16</sub> H <sub>32</sub> O <sub>2</sub>
37	31.669	<b>34.85</b>	299.32	C <sub>12</sub> H <sub>24</sub> O <sub>2</sub>

**Table 2.** GC-MS analysis of *W. somnifera* grown at elevated temperature (35-36 °C)

No	RT (min)	Area %	Molecular mass (g)	Molecular Formula
1	7.024	1.67	92.14	C <sub>7</sub> H <sub>8</sub>
2	10.176	0.18	255.49	C <sub>17</sub> H <sub>37</sub> N
3	11.224	0.27	182.35	C <sub>13</sub> H <sub>26</sub>
4	11.464	0.46	60.10	C <sub>2</sub> H <sub>8</sub> N <sub>2</sub>
5	12.219	0.17	204.36	C <sub>15</sub> H <sub>24</sub>
6	12.345	1.36	84.14	C <sub>5</sub> D <sub>5</sub> N
7	12.923	1.08	141.21	C <sub>8</sub> H <sub>15</sub> NO
8	14.428	0.06	142.20	C <sub>8</sub> H <sub>14</sub> O <sub>2</sub>
9	14.914	1.72	105.14	C <sub>7</sub> H <sub>7</sub> N
10	15.309	1.21	137.14	C <sub>7</sub> H <sub>7</sub> NO <sub>2</sub>
11	15.67	0.04	88.11	C <sub>4</sub> H <sub>8</sub> O <sub>2</sub>
12	15.881	0.27	163.38	C <sub>2</sub> HCl <sub>3</sub> O <sub>2</sub>
13	16.041	0.65	164.04	C <sub>3</sub> HF <sub>5</sub> O <sub>2</sub>
14	16.413	0.13	182.26	C <sub>11</sub> H <sub>18</sub> O <sub>2</sub>
15	16.648	0.39	85.15	C <sub>5</sub> H <sub>11</sub> N
16	16.991	<b>3.46</b>	154.25	C <sub>10</sub> H <sub>18</sub> O
17	17.363	0.76	80.13	C <sub>6</sub> H <sub>8</sub>
18	18.038	0.77	79.10	C <sub>5</sub> H <sub>5</sub> N

19	18.428	1.12	82.14	C <sub>6</sub> H <sub>10</sub>
20	18.628	1.64	122.12	C <sub>7</sub> H <sub>6</sub> O <sub>2</sub>
21	19.429	<b>4.49</b>	127.18	C <sub>7</sub> H <sub>13</sub> NO
22	19.686	0.61	326.74	C <sub>10</sub> H <sub>30</sub> O <sub>3</sub> Si <sub>4</sub>
23	20.259	1.15	72.11	C <sub>4</sub> H <sub>8</sub> O
24	21.071	0.4	296.62	C <sub>8</sub> H <sub>24</sub> O <sub>4</sub> Si <sub>4</sub>
25	23.011	3.02	127.18	C <sub>7</sub> H <sub>13</sub> NO
26	24.567	3.42	72.06	C <sub>3</sub> H <sub>4</sub> O <sub>2</sub>
27	24.962	0.22	503.07	C <sub>14</sub> H <sub>42</sub> O <sub>7</sub> Si <sub>7</sub>
28	25.46	0.23	168.23	C <sub>10</sub> H <sub>16</sub> O <sub>2</sub>
29	27.045	0.04	298.51	C <sub>19</sub> H <sub>38</sub> O <sub>2</sub>
30	27.24	<b>4.43</b>	223.31	C <sub>13</sub> H <sub>21</sub> NO <sub>2</sub>
31	27.497	1.88	158.20	C <sub>11</sub> H <sub>10</sub> O
32	28.012	0.08	92.14	C <sub>7</sub> H <sub>8</sub>
33	30.753	0.51	136.19	C <sub>9</sub> H <sub>12</sub> O
34	31.663	1.34	126.11	C <sub>6</sub> H <sub>6</sub> O <sub>3</sub>

**Table 3.** Compound presence in both ambient and elevated temperatures

No	RT (min)	Area%	Molecular mass	Molecular Formula
1	14.737	0.06	62.03	H <sub>2</sub> CO <sub>3</sub>
2	5.948	0.31	61.47	ClCN
3	9.742	0.08	60.05	CH <sub>3</sub> COOH
4	11.704	<b>0.26</b>	196.29	C <sub>12</sub> H <sub>20</sub> O <sub>2</sub>
5	11.950	<b>0.30</b>	136.20	C <sub>8</sub> H <sub>12</sub> N <sub>2</sub>
6	13.621	0.23	154.25	C <sub>10</sub> H <sub>18</sub> O
7	10.182	0.12	44.05	C <sub>2</sub> H <sub>4</sub> O
8	12.917	<b>0.53</b>	85.15	C <sub>5</sub> H <sub>11</sub> N
9	17.896	0.22	139.20	C <sub>8</sub> H <sub>13</sub> NO
10	20.688	0.07	108.14	C <sub>7</sub> H <sub>8</sub> O
11	17.495	0.07	128.17	C <sub>10</sub> H <sub>8</sub>

#### DECLARATION OF HONOR

We declare on our honor that our research are not fake and make up.

#### AI ASSISTANCE DISCLOSURE

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The authors used [ChatGPT/GPT-5] to improve the clarity and readability of the manuscript. The authors carefully reviewed and edited the content to ensure accuracy and take full responsibility for the final text.

## REFERENCES

- Attanayake, A.M.S.U.M., Banu, A., De Silva, C.S., De Silva, S., & Gunasekara, H.K.L.K.** (2025). Comparative GC-MS analysis of *Bacopa minnieri* grown at high temperature. *Asian Journal of Pharmacognosy*, 9(1):21-28. eISSN-0128-1119. Asian Society of Pharmacognosy. DOI: <http://dx.doi.org/10.71221/ajp.09103>
- Bitá, C., & Gerats, T.** (2013). Plant tolerance to high temperature in a changing environment: scientific fundamentals and production of heat stress-tolerant crops. *Frontiers in Plant Science, Volume 4-2013*. <https://doi.org/10.3389/fpls.2013.00273>
- Filipović, V., Ugrenović, V., Popović, V., Dimitrijević, S., Popović, S., Aćimović, M., Dragumilo, A., & Pezo, L.** (2023). Productivity and flower quality of different pot marigold (*Calendula officinalis* L.) varieties on the compost produced from medicinal plant waste. *Industrial Crops and Products*, 192, 116093. <https://doi.org/10.1016/j.indcrop.2022.116093>
- Gupta, K. K., Aneja, K. R., & Rana, D.** (2016). Current status of cow dung as a bioresource for sustainable development. *Bioresources and Bioprocessing*, 3, 1–11. <https://doi.org/10.1186/s40643-016-0105-9>
- Ingkaninan, K., Chuenchom, K., Temkitthawon, P., Yuyaem, T., & Thongnoi, W.** (2003). Screening for acetylcholinesterase inhibitory activity in plants used in Thai traditional rejuvenating and neurotonic remedies. *Journal of Ethnopharmacology*, 89(2–3), 261–264. <https://doi.org/10.1016/j.jep.2003.08.008>
- IPCC.** (2007). *Summary for policymakers*. In: S. Solomon, D. Qin, M. Manning, M. Marquis, K. Averyt, et al (Eds.), *Climate change 2007: The physical science basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change* (pp. 2–22). Cambridge University Press. <https://www.ipcc.ch/report/ar4/wg1/>
- Jha, U. C., Warburton, M. L., Nayyar, H., Chatti, D., Ghatak, A., Siddique, K. H. M., & Prasad, P. V. V.** (2025). Heat stress-induced metabolomic shifts in chickpea (*Cicer arietinum* L.) flowers insights from contrasting genotypes. *Scientific Reports*, 15(1), 34821. <https://doi.org/10.1038/s41598-025-21697-w>
- Kumar, A., Dadhech, A., Mondal, K., & Yadav, M.** (2022). Relevance of Ashwagandha (*Withania somnifera*) root extracts for good health and stamina: A review of recent advancements. *The Journal of Phytopharmacology*, 11, 217–220. <https://doi.org/10.31254/phyto.2022.11314>
- Langade, D., Kanchi, S., Salve, J., Debnath, K., & Ambegaokar, D.** (2019). Efficacy and Safety of Ashwagandha (*Withania somnifera*) Root Extract in Insomnia and Anxiety: A Double-blind, Randomized, Placebo-controlled Study. *Cureus*, 11(9), 57–97. <https://doi.org/10.7759/cureus.5797>
- Mikulska, P., Malinowska, M., Ignacyk, M., Szustowski, P., Nowak, J., Pesta, K., Szelaę, M., Szklanny, D., Judasz, E., Kaczmarek, G., Ejiohuo, O. P., Paczkowska-Walendowska, M., Gościński, A., & Cielecka-Piontek, J.** (2023). Ashwagandha (*Withania somnifera*)-Current Research on the Health-Promoting Activities: A Narrative Review. *Pharmaceutics*, 15(4), 1057. <https://doi.org/10.3390/pharmaceutics15041057>
- Mirjalili, M. H., Moyano, E., Bonfill, M., Cusido, R. M., & Palazón, J.** (2009). Steroidal Lactones from *Withania somnifera*, an Ancient Plant for Novel Medicine. *Molecules*, 14(7), 2373–2393. <https://doi.org/10.3390/molecules14072373>
- National Institutes of Health [NIH].** (2023). *Ashwagandha: Health professional fact sheet*. <https://ods.od.nih.gov/factsheets/Ashwagandha-HealthProfessional/>

- Paul, S., Chakraborty, S., Anand, U., Dey, S., Nandy, S., Ghorai, M., Saha, S. C., Patil, M. T., Kandimalla, R., Proćków, J., & Dey, A.** (2021). *Withania somnifera* (L.) Dunal (Ashwagandha): A comprehensive review on ethnopharmacology, pharmacotherapeutics, biomedical and toxicological aspects. *Biomedicine & Pharmacotherapy*, 143, 112175.  
<https://doi.org/https://doi.org/10.1016/j.biopha.2021.112175>
- Ruelland, E., & Zachowski, A.** (2010). How plants sense temperature. *Environmental and Experimental Botany*, 69(3), 225–232.  
<https://doi.org/https://doi.org/10.1016/j.envexpbot.2010.05.011>
- Silva, C. S. D., Weatherhead, E. K., Knox, J. W., & Rodrihuez-Diaz, J. A.** (2007). Redicting the impacts of climate change-A case study of paddy irrigation water requirements in Sri Lanka. *Journal of Agricultural Water Management*, 93, 19–29.  
<https://doi.org/https://doi.org/10.1016/j.agwat.2007.06.003>
- Singh, A., & Mina, U.** (2025). Temperature stress and its effects on phytochemical dynamics and antioxidant activity in *Withania somnifera* (L). dunal. *Physiology and Molecular Biology of Plants*, 31(4), 675–692. <https://doi.org/10.1007/s12298-025-01594-2>
- Singh, N., Bhalla, M., de Jager, P., & Gilca, M.** (2011). An overview on Ashwagandha: A Rasayana (rejuvenator) of Ayurveda. *African Journal of Traditional, Complementary and Alternative Medicines*, 8(5 Suppl), 208–213.  
<https://doi.org/https://doi.org/10.4314/ajtcam.v8i5S.9>
- Yang, L., Wen, K.-S., Ruan, X., Zhao, Y.-X., Wei, F., & Wang, Q.** (2018). Response of Plant Secondary Metabolites to Environmental Factors. *Molecules*, 23(4).  
<https://doi.org/10.3390/molecules23040762>