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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 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: global warming, temperature stress

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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* roots are withanolides and withanosides, steroidal lactones that include withaferin A, withanolide A, withanolide D, withanone, withanolide B, 12-deoxywithastramonolide, and withanoside IV–VI. However, metabolomic profiling shows that the roots also contain other constituents, including alkaloids, phenolic metabolites, phytosterols, and fatty acid (Elghazaly et al., 2025; Jayathilaka et al., 2025; Trivedi et al., 2017). 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 temperatures influence Ashwagandha's root phytochemical dynamics is increasingly important under climate change, as prolonged periods of thermal stress can significantly alter the plant's chemistry and therapeutic

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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) used for profiling volatile and semi-volatile metabolites and assessing effect of environmental factors on chemical diversity in roots.

This study investigates the impact of temperature on the secondary-metabolite profile of *W. somnifera* roots grown under controlled conditions. The selected elevated temperature range exceeds the typical ambient conditions (27–32 °C) and represents projected increases in temperature under future climate scenarios (IPCC, 2007; Silva et al., 2007). In this study, elevated temperature is therefore defined as a heat stress condition beyond the optimal physiological range, where metabolic and biochemical responses are expected to occur. Prolonged exposure to such thermal stress can significantly alter plant chemistry and therapeutic potential (Jha et al., 2025). By comparing GC-MS profiles of plants cultivated at ambient and elevated temperatures, we aim to determine whether thermal stress alters metabolite profile, and abundance. 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 (T1; 27°C -32 °C) in a naturally ventilated plant house or under elevated temperature conditions (T2; 35°C -36 °C) inside a temperature regulated poly tunnel. 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 (T1; 27°C –32 °C) with compost treatment. Representative samples illustrate uniform vegetative growth prior to root harvest for GC–MS analysis. (T1: Ambient temperature, M1: Compost application, and R: Replicate)

Extr

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 (≥ 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 µL 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 (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%;

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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 (Table 2; Figure 2), 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 (Table 3), including MW 136.20 ($C_8H_{12}N_2$), 85.15 ($C_5H_{11}N$), and 61.47 (ClCN). 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.

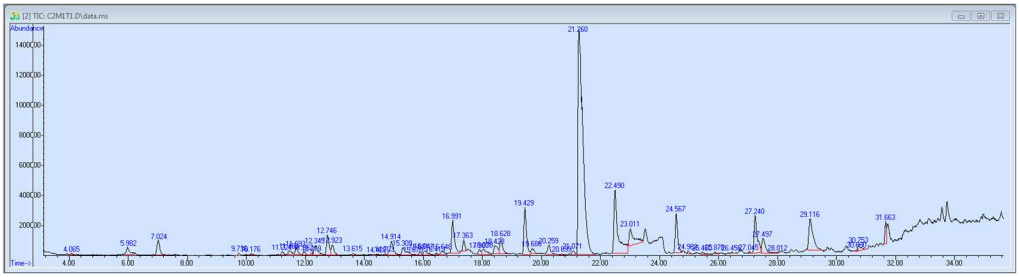

Figure 2. Chromatogram of Ashwagandha (*W. somnifera*) root extract grown under ambient temperature conditions (27-32 °C).

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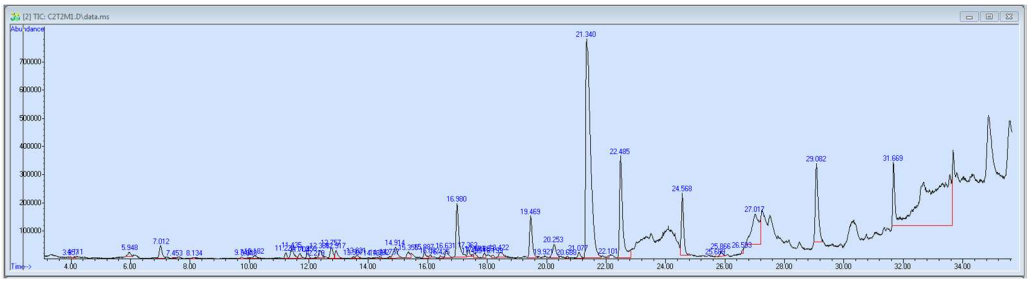

Figure 3. Chromatogram of Ashwagandha (*W. somnifera*) root extract grown under elevated temperature stress (35-36 °C).

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	$C_6H_{11}NO_4$
2	4.111	0.06	62.03	H_2CO_3
3	7.012	0.81	88.11	$C_4H_8O_2$
4	7.453	0.05	94.11	$C_5H_6N_2$
5	8.134	0.1	123.20	$C_8H_{13}N$
6	9.948	0.08	424.79	$C_{29}H_{60}O$
7	10.182	0.17	70.09	C_4H_6O
8	11.224	0.31	228.42	$C_{15}H_{32}O$
9	11.435	0.74	56.06	C_3H_4O
10	12.219	0.04	135.13	$C_5H_5N_5$
11	12.368	0.5	101.15	$C_5H_{11}NO$
12	12.757	0.74	152.23	$C_{10}H_{16}O$
13	12.917	0.53	218.21	$C_{11}H_{10}N_2O_3$
14	13.507	0.11	102.13	$C_5H_{10}O_2$
15	14.188	0.03	254.41	$C_{16}H_{30}O_2$
16	14.382	0.11	59.11	C_3H_9N
17	14.914	1.04	84.16	C_6H_{12}
18	15.355	0.42	137.14	$C_7H_7NO_2$
19	15.887	0.37	238.46	$C_{17}H_{34}$

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20	16.082	0.13	98.10	$C_5H_6O_2$
21	16.425	0.12	96.17	C_7H_{12}
22	16.631	0.52	85.10	C_4H_7NO
23	16.98	3.64	154.25	$C_{10}H_{18}O$
24	17.363	0.33	80.13	C_6H_8
25	17.598	0.26	128.17	$C_{10}H_8$
26	18.199	0.13	112.09	$C_4H_4N_2O_2$
27	18.422	0.51	204.36	$C_{15}H_{24}$
28	19.469	2.66	123.11	$C_6H_5NO_2$
29	19.921	0.02	125.14	$C_5H_7N_3$
30	20.253	1.19	100.16	$C_6H_{12}O$
31	21.077	0.37	112.17	$C_7H_{12}O$
32	**21.34**	**25.05**	**127.18**	**$C_7H_{13}NO$**
33	22.101	0.07	100.12	$C_5H_8O_2$
34	24.568	3.47	72.06	$C_3H_4O_2$
35	26.593	0.11	214.35	$C_{13}H_{26}O_2$
36	**27.017**	**5.61**	**256.43**	**$C_{16}H_{32}O_2$**
37	**31.669**	**34.85**	**299.32**	**$C_{12}H_{24}O_2$**

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_7H_8
2	10.176	0.18	255.49	$C_{17}H_{37}N$
3	11.224	0.27	182.35	$C_{13}H_{26}$
4	11.464	0.46	60.10	$C_2H_8N_2$
5	12.219	0.17	204.36	$C_{15}H_{24}$
6	12.345	1.36	84.14	C_5D_5N
7	12.923	1.08	141.21	$C_8H_{15}NO$
8	14.428	0.06	142.20	$C_8H_{14}O_2$
9	14.914	1.72	105.14	C_7H_7N
10	15.309	1.21	137.14	$C_7H_7NO_2$
11	15.67	0.04	88.11	$C_4H_8O_2$
12	15.881	0.27	163.38	$C_2HCl_3O_2$
13	16.041	0.65	164.04	C_3HF_5O2
14	16.413	0.13	182.26	$C_{11}H_{18}O_2$
15	16.648	0.39	85.15	$C_5H_{11}N$

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16	**16.991**	**3.46**	**154.25**	**$C_{10}H_{18}O$**
17	17.363	0.76	80.13	C_6H_8
18	18.038	0.77	79.10	C_5H_5N
19	18.428	1.12	82.14	C_6H_{10}
20	18.628	1.64	122.12	$C_7H_6O_2$
21	**19.429**	**4.49**	**127.18**	**$C_7H_{13}NO$**
22	19.686	0.61	326.74	$C_{10}H_{30}O_3Si_4$
23	20.259	1.15	72.11	C_4H_8O
24	21.071	0.4	296.62	$C_8H_{24}O_4Si_4$
25	23.011	3.02	127.18	$C_7H_{13}NO$
26	24.567	3.42	72.06	$C_3H_4O_2$
27	24.962	0.22	503.07	$C_{14}H_{42}O_7Si_7$
28	25.46	0.23	168.23	$C_{10}H_{16}O_2$
29	27.045	0.04	298.51	$C_{19}H_{38}O_2$
30	**27.24**	**4.43**	**223.31**	**$C_{13}H_{21}NO_2$**
31	27.497	1.88	158.20	$C_{11}H_{10}O$
32	28.012	0.08	92.14	C_7H_8
33	30.753	0.51	136.19	$C_9H_{12}O$
34	31.663	1.34	126.11	$C_6H_6O_3$

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_2CO_3
2	**5.948**	**0.31**	**61.47**	**ClCN**
3	9.742	0.08	60.05	CH_3COOH
4	11.704	0.26	196.29	$C_{12}H_{20}O_2$
5	**11.950**	**0.30**	**136.20**	**$C_8H_{12}N_2$**
6	13.621	0.23	154.25	$C_{10}H_{18}O$
7	10.182	0.12	44.05	C_2H_4O
8	**12.917**	**0.53**	**85.15**	**$C_5H_{11}N$**
9	17.896	0.22	139.20	$C_8H_{13}NO$
10	20.688	0.07	108.14	C_7H_8O

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11	17.495	0.07	128.17	$C_{10}H_8$

DISCUSSION

Temperature stress (35°C –36 °C) altered metabolite distribution in *W. somnifera* roots without disrupting core pathways. Under ambient conditions, a few dominant metabolites accounted ~65% of total chromatographic area indicated preferential allocation toward lipid-associated and nitrogen-containing compounds. Elevated temperature reduced this dominance and redistributed metabolites across a broader spectrum, consistent with stress-induced membrane remodeling and shifts in metabolic flux (Bita & Gerats, 2013; Ruelland & Zachowski, 2010). The persistence of nitrogen-containing molecules suggests stability of amino acid and alkaloid related pathways, supporting the observation that environmental stress reshapes, rather than suppresses, specialized metabolism (Yang et al., 2018). Given that GC–MS primarily detects volatile and semi-volatile compounds (Attanayake et al., 2025), Due to resource limitations, a relative metabolite profiling approach was adopted instead of absolute quantification using external standards. Key bioactive compounds as withanolides require LC–MS/HPLC approaches (Mirjalili et al., 2009) and should be targeted in future studies. 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 alters volatile metabolite composition of *W. somnifera* roots, with elevated temperature reducing metabolite dominance and promoting a broader distribution of compounds, indicating metabolic rebalancing without loss of core biosynthetic capacity. These findings are particularly relevant to Sri Lanka, where *W. somnifera* is widely used in Ayurvedic medicine. Climate-driven temperature increases may lead to variability in phytochemical composition, potentially affecting the consistency, quality, and therapeutic reliability of herbal formulations. Understanding these changes can support the standardization of raw materials, improve quality control in herbal drug production, and ensure consistent treatment outcomes within the healthcare system. Future studies should quantify the main withanolides withanosides, and integrate multi-omics approaches to assess how temperature-induced changes influence pharmacological efficacy.

DECLARATION OF HONOR

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

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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.

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