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Research paper

Comparative GC-MS analysis of *Bacopa monnieri* grown at high temperature

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ABSTRACT

Context: *Bacopa monnieri* (L.) Wettst. is traditionally used as a nerve tonic for mental disorders in Ayurvedic medicine in India and Sri Lanka. However, due to global warming, natural habitats habitat of this plant is threatened. ***Objective:*** to examine changes in secondary metabolites in *B. monnieri* with increased temperature. ***Methods:*** plants were grown inside a temperature regulated poly tunnel at 34°C -35 °C and ambient temperature plant house at 27 °C - 32° GC-MS was used to analyze difference in secondary metabolites. ***Results:*** A compound with the molecular formula of $C_{20}H_{40}O$ corresponding to phytol A is the major constituent Plants grown at higher temperature produce substances that are no present at ambient temperature and heat influence the yield of secondary metabolite production. ***Conclusion:*** increased temperature affects the composition of *B. monnieri.*

Keywords: Temperature stress, global warming

INTRODUCTION

Sri Lanka is renowned as a worldwide hub of biodiversity, due to its rich and varied ecosystems teeming with life (Dharmadasa, 2016). Different perspectives about the side effects of modern medicine (Gurib, 2006) increased the usage of plants and penetrated much of Sri Lankan and Indian culture, particularly in traditional health care. There are four different practices of traditional medical system in Sri Lanka such as Siddha, Unani, Ayurveda, and Deshiya Chikitsa Sidda medicine is a form of traditional medicine originating in Southern India and it is one of the oldest systems of Medicine in India. Unani medicine is Perso-Arabic traditional medicine as practiced in Muslim culture in South Asia and modern-day Central Asia. Ayurveda medicine is

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as alternative medicine system with historical roots in the Indian Subcontinent. It is heavily practiced throughout India, Sri Lanka and Nepal, where as much as 80% of the population repot using ayurveda as their sole medical system. Ayurveda relies on a comprehensive assessment of a person's physical, mental and emotional state as well as their individual constitution (Prakrithi). Deshiya Chikitsa is a purely native kind of medicine that has been practicing since pre-historic period in Sri Lanka (Weragoda, 1980). Medicinal plants have proven to be a valuable reservoir of therapeutic compounds, with numerous modern drugs being either directly derived from natural plant sources or developed from their derivatives (Mohotti et al*.,* 2020).

Among various medicinal Ayurvedic herbs, *Bacopa monnieri* (L.) Wettst. or called "Lunuwila" in Sinhala language of Sri Lanka and "Brahmi in Sanskrit in India and also known as water hyssop is considered as herb of grace and belonging to the family Plantaginaceae. "Lunuwila" is used as a brain booster by amassing information evolving through experience over years and years because it acts as a rejuvenator for the brain and nervous system (Fatima et al., 2022). "Lunuwila" is used in traditional medicine to treat various nervous disorders, as a brain tonic to enhance memory development, learning and concentration, and to provide relief with anxiety; it is also used in digestive complaints, for skin disorders and as antiepileptic, antipyretic, and analgesic (Jain et al, 2017). Historically, the use of *B. monnieri* dates to approximately 6th century AD. It has been reported for various pharmacological activities. Originally, these plants were known to grow wildly and naturally. Unfortunately, many of these plants are threatened by the hazard of extinction.

Medicinal plants are especially important in modern civilization to obtain natural active substances, known as secondary metabolites. Secondary metabolites of medical plants are the material basis of their clinically curative effects. They are also important indicators for evaluating the quality of medicinal materials (Li et al., 2020). Recognizing the growth responses of "Lunuwila" to temperature stress and Similarly, qualitative, and quantitative identification of biochemical compounds is important since it plays a major role in pharmaceutical production. Therefore, this research focused on GC-MS analysis of the dry shoots and roots of the plants grown under ambient temperature and global warming induced temperature stress

METHODS

Plant material

Authenticated healthy plants were obtained from Medicinal Plant Garden, Ganewatta, Sri Lanka and vegetatively propagated in a nursery to obtain plantlets for the experiment. For the nursery media, a mixture of 3 parts of reddish-brown earth (RBE) soil, 1 part coir dust, and 1 part compost was used. Pot soil water capacity was maintained using continuous irrigation until the pot becomes saturated. The nursery potting medium was saturated by fully watering it until water starts draining from the bottom. The nursery pots were 20 cm in diameter and 25 cm in height. Plants were grown under ambient temperature (27°C -32°C) in plant house and increased temperature stress (35°C -36 °C) in temperature regulated poly tunnel with 100% compost (1 kg) with soil mixture (9 kg).

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Extraction

The whole plant of *B. monnier*i was dried under shade at room temperature and then powdered using a mill. The shade dried plant powder was soaked with methanol (1:10 ratio). The flask was covered with aluminum foil to avoid evaporation and then kept for 48 hours in a 50-rpm shaker incubator at room temperature. After 48 hours the solution was filtered by using Whatman filter paper No.1 and the filtrate was collected in a beaker. Then, the filtrate was kept in an incubator at 37 °C to evaporate the solvent. The prepared extract was then stored at 4 °C for further use (Ingkaninan et al*.,* 2003).

GC-MS

The GC-MS analysis of both extracts was performed using GCMS QP-2010 Plus Shimadzu Company instrument equipped with H5 column. Initially, the oven temperature was maintained at 80 °C for 2 min. and the temperature gradually increased up to 250 °C at 5 min. and 1.0 µL of sample was injected for analysis. Helium was the carrier gas. The flow rate of helium gas was 1.2 ml/min. The sample injector and mass transfer line temperature were set at 250 °C and split ratio is 10 throughout the experiment periods. The ionization mass spectroscopic analysis was done with 70 eV. Mass spectra were recorded across the range of 40 to 600 m/z for the duration of 50 min (Shibula, 2015).

RESULTS

GC-MS analysis of sample grown at ambient temperature

Fifty-six peaks which indicated the presence of 56 compounds were observed (Table 1, Figure 1). The major peak at Rt: 32.837 mins corresponded to a substance with a molecular formula of $C_{20}H_{40}O$ corresponding to phytol as per the database. A second major peak at Rt 29.083 corresponding to the molecular formula of C_2H_7NO was observed.

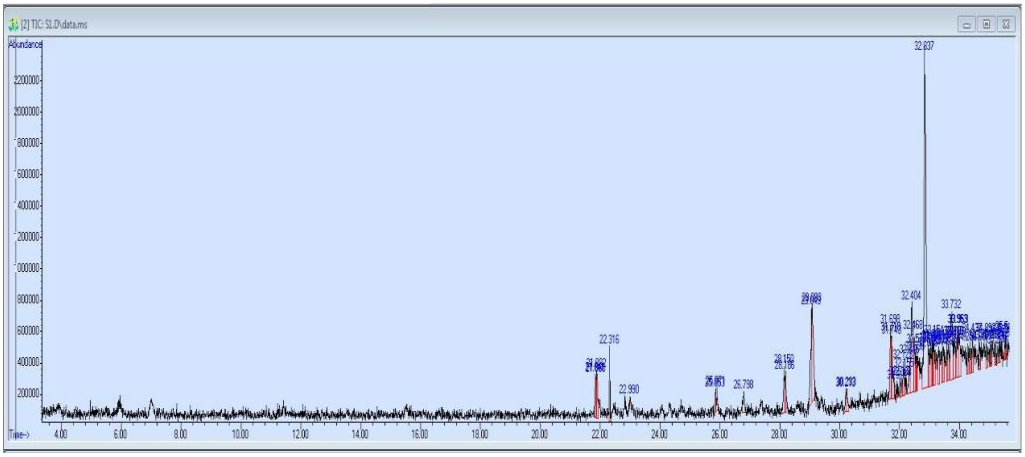

Figure 1. GC-MS analysis of *B. monnieri* grown at ambient temperature

Table 1 GC-MS analysis of *B. monnieri* grown at ambient temperature

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No	RT	Area%	Compound name suggested by the data base	Molecular formula
1	21.841	1.22	Bicyclo [3.1.1] heptane, 2,6,6-trimethyl	$C_{10}H_{18}$
2	21.860	0.89	Neophytadiene	$C_{20}H_{38}$
3	21.882	1.83	5,6-Epoxydecane	$C_{10}H_{20}O$
4	22.316	0.61	Noraporphin-7-one, 4,5,6,6a-tetrad ehydro-2-hydroxy-1,3-dimethoxy-, acetate (ester)	$C_{15}H_{17}NO_5$
5	25.871	0.95	1-methyl- 1-[[1',3'S-Dihydroxy-2'R-butoxy] methyl] thymine, 1'-diphenyl phosphate Fumaric acid	$C_{26}H_{33}N_2O_{12}P$
6	26.798	0.99	2,5-Diethylphenol , 3-Methoxyacetophenone	$C_{10}H_{14}O$, $C_9H_{10}O_2$
7	28.150	1.68	Succinic acid, 2-chloro-4-methylphenyl isohexyl ester	$C_{17}H_{23}ClO_4$
8	28.186	1.36	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	$C_6H_8O_4$
9	**29.083**	**4.73**	**Methanamine, N-methoxy**	**C_2H_7NO**
10	30.213	1.04	2H-1-Benzopyran-2-one	$C_9H_2O_2$
11	30.213	1.04	Butanoic acid	$C_4H_8O_2$
12	30.233	0.81	1-[2-Acetoxyethyl]-4-[4-aminobenzoyl] piperazine 1H-Pyrrole	$C_{18}H_{28}N_4O_2$
13	31.698	2.26	Dodecanoic acid	$C_{12}H_{24}O_2$
`14	31.718	1.53	Pentadecanoic acid	$C_{15}H_{30}O_2$
15	31.743	2.25	Tridecanoic acid	$C_{13}H_{26}O_2$
16	31.917	0.16	Propane, 1,1-diethoxy- Silane, triethyl	$C_{10}H_{24}O_2Si$
17	32.083	0.49	Bis(n-propylthio) methane	$C_7H_{16}S_2$
18	32.013	1.13	2H-1,4-Benzoxazin-3(4H)-one	$C_8H_7NO_2$
19	32.345	1.51	N- [4-[[(4-aminopheny l)sulfonyl]amino]-2-methoxyphenyl]	$C_{13}H_{14}N_2O_3S$
20	32.404	6.25	1,6-Anhydro-2,4-dideoxy-. beta. -D-arabo-hexopyranose	$C_4H_8O_4$
21	32.530	0.54	Acetamide derivates	CH_3CONH_2
22	32.576	0.96	Silane derivates	SiH_4
23	**32.837**	**22.92**	**Phytol**	**$C_{20}H_{40}O$**
24	33.093	0.70	8a-ethoxy-3a,3b,7a,8a-tetrahydro-2,2,5,5-tetramethyl	$C_{12}H_{22}O_2$
25	33.303	1.227	1,3-Dioxolane	$C_3H_6O_2$
26	33.28	1.428	4,7,10,13,16-Pentaoxanonadeca-1,18-diene	$C_{14}H_{26}O_5$
27	33.468	0.859	1,4,7,10,13-Pentaoxacyclopentadecane	$C_{10}H_{20}O_5$
28	33.575	1.376	Diglycolic acid	$C_4H_6O_5$
29	33.629	1.074	1,3,4-Trimethoxy-butan-2-ol	$C_7H_{16}O_4$
30	33.732	5.121	2-(2-Methoxyethoxy) acetic acid	$C_5H_{10}O_4$
31	33.881	1.164	2,5,8,11,14Pentaoxahexadecan-16-ol	$C_{11}H_{24}O_6$
32	33.953	1.731	Tetracyclo (6.2.1.0(2,7).0(3,5)) undecane	$C_{11}H_{16}$
33	34.258	0.597	3,6,9,12-Tetraoxatetradecan-1-ol	$C_{10}H_{22}O_5$
34	33.817	2.33	2-(2-{2-[2-(2-Methoxy-ethoxy)-ethoxy]-ethoxy}-ethoxy)-ethanol	$C_9H_{20}O_6$
35	35.194	0.365	1-(3-Ethoxy-4-methoxyphenyl)-2-(methylsulfonyl) ethanamine	$C_9H_{15}NO_3S$
36	35.26	0.371	2,5,8,11,14,17-Hexaoxanonadecan-19-ol	$C_{18}H_{38}O_7$
37	35.339	0.263	Propane, 1,2,3-trimethoxy	$C_6H_{14}O_3$
38	35.453	0.354	1-Methoxy-5-dimethyl(ethyl)silyloxy-3-phenylpentane	$C_{16}H_{28}O_2Si$
39	35.613	0.288	3,6,9,12,15-Pentaoxanonadecan-1-ol -	$C_{14}H_{30}O_6$

GC-MS analysis of sample grown at high temperature

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Sixty six peaks which indicated the presence 66 compounds were observed (Figure 2, Table 2). The major peak at Rt: 32.84 mins was a substance with a molecular formula of $C_{20}H_{40}O$ corresponding to phytol as per the database, but compared to ambient temperature it was in present in lesser amount Table 2 provide a list of 15 compounds that were not found at ambient temperature, of which major peak at Rt 7 mins with the molecular formula of $C_{14}H_{14}ClO_4$ corresponding to hydroxy(4-benzyloxy-3-chlorophenyl) acetic acid according to the database.

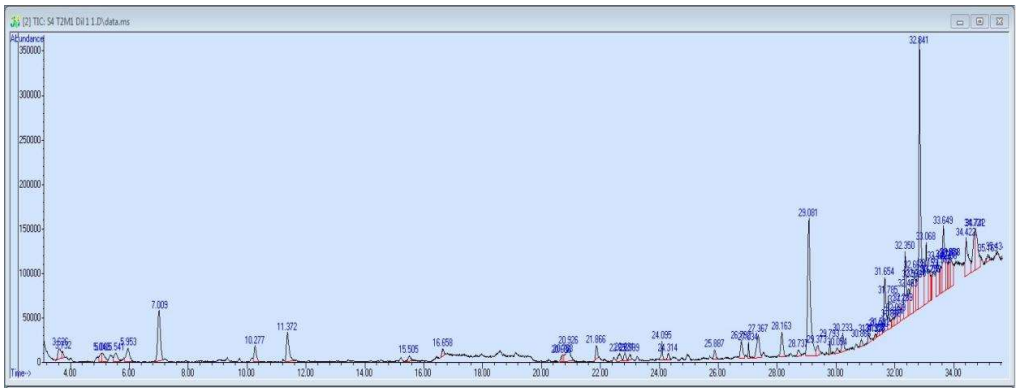

Figure 2. GC-MS analysis of *B. monnieri* grown at high temperature

Table 2 GC-MS analysis of *B. monnieri* grown at high temperature (compounds present at normal temperature not included)

No	RT	Area %	Compound name suggested by the data base	Molecular Formula
1	3.62	0.85	3,6-Diketohept-1-ene,	$C_7H_{10}O_2$
2	5.08	0.75	1-Butanamine, 2-methyl-N-(2-methyl butylidene	$C_{10}H_{21}N$
3	**7.00**	**4.00**	**Hydroxy(4-benzyloxy-3-chlorophenyl) acetic acid**	**$C_{14}H_{14}ClO_4$**
4	11.37	2,24	Benserazide	$C_{10}H_{15}N_3O_5$
5	15.50	0.48	Benzene acetaldehyde	C8H8O
6	16.65	0.47	4-Ethylbenzoic acid, 3-methylbutyl Ester	$C_{14}H_{20}O_2$
7	21.86	0.66	18-Nonadecen-1-ol	$C_{19}H_{38}O$
8	22.99	0.29	1-Dodecyne, 1-Tridecyne, and 5-Undecyne	$C_{12}H_{22}$, $C_{13}H_{24}$, $C_{11}H_{20}$
9	26.79	1.14	2-Methoxy-4-vinylphenol	$C_9H_{10}O_2$
10	26.79	1.14	2-Methoxy-1,3,4-trimethylbenzene	$C_{10}H_{14}O$
11	27.03	0.41	3,4-Dihydroxyphenylglycol	$C_8H_{10}O_3$
12	27.36	1.94	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$
13	28.73	0.43	1,2,4-triazole derivatives	$C_2H_3N_3$
14	28.73	0.43	Butanoic acid	$C_4H_8O_2$
15	29.37	0.71	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl	$C_{10}H_{16}O_2$

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DISCUSSION

The production of secondary metabolites in medicinal plants is significantly influenced by temperature variations. Studies have shown that both elevated temperatures (heat stress) and low temperatures (cold stress) can lead to an increase in the production of secondary metabolites (Sun et al*.,* 2015). Environmental factors such as temperature, light intensity, photoperiod, and soil fertility play a crucial role in influencing the biosynthesis of secondary metabolites in plants (Sun et al*.,* 2015). Increased temperature can lead to significant changes in plant metabolism. High temperatures can cause an increase in the production of certain metabolites as part of the plant's stress response mechanism (Wahid et al. 2007). Plants have the remarkable ability to produce a diverse array of secondary metabolites, such as phenolics and terpenes, which are known for their antioxidant properties (Erwin et al., 2019). These compounds play a crucial role in combating oxidative damage, particularly under stress conditions like temperature stress, where plants may increase their production to mitigate the effects of heat stress (Barku,2019*).* The upregulation of secondary metabolites with antioxidant properties, such as 3,6,9,12-Tetraoxatetradecan-1-ol and 2-(2- {2-2-(2-Methoxy-ethoxy)-ethoxy-ethoxy}-ethoxy)-ethanol, could be attributed to the response to temperature stress (Raduan et al., 2022). For instance, the levels of certain secondary metabolites like phenolics, flavonoids, and terpenoids increase under stress conditions (Bardehji, 2023). Additionally, the synthesis of secondary metabolites is influenced by environmental factors such as temperature, water availability, and CO_2 levels (Mena et al*.,* 2014).

Compounds like phytol are associated with lipid metabolism and membrane stability. Under temperature stress, the lipid composition of cellular membranes can change to maintain membrane fluidity and function. The observed decrease in phytol under temperature stress may be related to these adaptive changes in lipid metabolism (Upchurch, 2008). Under temperature stress, plants utilize compounds like butanoic acid to help maintain membrane stability by preserving the integrity and fluidity of cell membranes, thus safeguarding plant cells from damage (Hancock et al*.,* 2013). Moreover, organic acids contribute to osmotic balance, aiding in osmotic adjustment to help plant cells retain water and maintain turgor pressure during stressful conditions (Montesinos-Navarro, 2024). Compounds like butanoic acid can also function as signaling molecules, activating stress response genes and enabling plants to better tolerate temperature fluctuations (Montesinos-Navarro, 2024). Additionally, the synthesis of secondary metabolites is dependent on factors like glucose metabolism, which can be affected by temperature changes, leading to alterations in the production of phenolic compounds. Furthermore, it has been observed that high temperatures and solar radiation levels contribute to the production of phenolic metabolites in certain plant species (Montesinos-Navarro, 2024). Increased temperature as an abiotic stress factor can have a considerable impact on the levels of secondary metabolites in plants (Upchurch, 2008). The presence or absence of specific secondary metabolites in medicinal plants is influenced by several factors including climate, season, and edaphic conditions (Montesinos-Navarro, 2024).

The changes in the amounts of secondary metabolites under different temperature conditions can be attributed to several physiological and biochemical responses of plants to temperature stress. Research has demonstrated that plants

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exhibit changes in metabolite production in response to different environmental conditions, including temperature fluctuations (Sun et al*.,* 2015). Phenolic compounds play a crucial role in plant defense mechanisms under stress conditions, with their biosynthesis being induced by temperature stress, leading to an increase in antioxidant compounds like flavonoids and phenolic acids (Chowdhary *et al.*, 2022; Muthusamy, 2024). This adaptive response helps plants mitigate oxidative stress; a common secondary stressor associated with abiotic stresses like high temperatures (Muthusamy, 2024). Research by Zheng *et al,* (2020) indicated that high temperatures can affect the concentrations of endogenous hormones in plants, with significant reductions in cytokinin and indole acetic acid concentrations observed under high-temperature conditions. This hormonal regulation in response to temperature changes could influence the biosynthesis or accumulation of polyether alcohols in plants grown in high-temperature settings.

CONCLUSION

Increase of temperature results in a change in the chemical composition of *B. monnieri*

DECLARATION OF CONFLICT OF INTEREST

No conflict of interest to declare.

DECLARATION OF HONOUR

We declare in our honor that our results are not fake and made up.

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