



SEM-EDX AND GC-MS ANALYSIS OF PHYTOCONSTITUENTS IN *Anisomeles heyneana* Benth. LEAVES FROM WESTERN GHATS (INDIA)

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ABSTRACT

The medicinal herb *Anisomeles heyneana* Benth is endemic to peninsular India. In current study the phytoconstituents in the leaves of *Anisomeles heyneana* were investigated. The plant material was collected from the Western Ghats in India. The phytochemicals were extracted from the leaves by preparing a methanol extract and analyzed using GC-MS and SEM-EDX. The GC-MS analysis of extract revealed the presence of 22 compounds in it. These compounds included phenolics, flavonoids, coumarins, glycosides, fatty acids, terpene and steroids. The predominant compounds in the leaves were 13, 27-cycloursan-3-one (100%), γ -sitosterol (82.04%) and n-hexadecanoic acid (34.08%). An energy-dispersive X-ray spectrometer (EDS) was employed to analyse the mass percentage of trace elements. Scanning electron microscopy showed the presence of carbon, oxygen, sodium, magnesium, aluminium, silicon, phosphorus, chlorine, calcium, magnesium, and iron. The carbon was the highest component (48.76 %) as compared with other minerals.

Keywords: *Anisomeles heyneana*, elemental analysis, GC-MS, SEM-EDX

INTRODUCTION

According to the World Health Organization (WHO), more than 80% of the world's population still relies primarily on herbal or plant-based medicines to treat a variety of diseases (Uritu *et al.*, 2018). About 70,000 plant species have been used ethnomedically worldwide. These are used as in folk medicine as pain-relieving medicines. The presence of bioactive compounds in plants play a crucial role in the advancement of phytotherapeutic treatment (Nasim *et al.*, 2022). Lamiaceae (al. Labiatae) is a widely distributed aromatic plant family used in traditional medicine and pharmacy. It includes 236 genera and 7000 species distributed worldwide and may also be referred to as the "mint family" (Chakrabartty *et al.*, 2022). A variety of bioactive chemicals, including alkaloids, flavonoids, terpenoids, saponins, phenols, and tannins are synthesized by different species of the Lamiaceae family. These compounds exhibit various biological properties as antioxidant, anticancer, antiinflammatory, and antimicrobial (Marchioni *et al.*, 2020). Members of the Lamiaceae family have been used as culinary herbs namely basil (*Ocimum* spp.), mint (*Mentha* spp.), sage (*Salvia officinalis*), marjoram (*Origanum majorana*). The aromatic essential oils extracted from these plants are used in the cosmetic, flavoring, pesticide, fragrance, perfume and pharmaceutical industries (Carovic-Stanko *et al.*, 2016).

The use of gas chromatography in combination with mass spectrometry (GC-MS) for phytochemical profiling has become an important method in the identification and quantification of

biologically active compounds in medicinal plants. The bioactive compounds isolated from the Lamiaceae family namely menthol, limonene, 1, 8-cineole, pulegone, carvone, β -caryophyllene, vincristine, reserpine, catharin and ajmalicin have antioxidant, anticancerous, antiinflammatory, antihypertensive, antiallergic, and antiviral properties (Karpinski, 2021; Bansal *et al.*, 2023). *A. indica* and *A. malabarica* exhibits bactericidal and fungicidal activities and are used in wound healing, skin cancer, antioxidants, anti-HIV and Helicobacter-induced inflammation, anti-inflammatory properties, hypertension, and suppression of tumor cell growth (Nasrin *et al.*, 2022).

Anisomeles heyneana Benth (also known as 'Western Hill Catmint') is endemic to regions of peninsular India and belongs to the Lamiaceae family (Sardesai and Yadav 2002). *A. heyneana* is native to northern Australia, Malaysia, and Southeast Asia. It is a 1.2-3.0 m tall shrub with a pleasant aromatic fragrance due to presence of volatile essential oils (Bean, 2015). The tiny flowers bloom in long cymes from October to November. The flowers look like the lobes of cow ears (Mutha *et al.*, 2021). Mutha *et al.* (2021) studied the presence of diterpenes in *A. heyneana* and investigated its potential against *Mycobacterium tuberculosis*. However, limited information is available on phytochemical studied on *Anisomeles heyneana* (Ushir *et al.*, 2010; Mutha *et al.*, 2021; Dhulap *et al.*, 2023). The aim of present study was to analyse the methanolic extract of *Anisomeles heyneana* leaves for various phytoconstituents and bioactive components using GC-MS and SEM-EDX analysis.

MATERIALS AND METHODS

Collection of plant material and preparation of methanolic extract

Anisomeles heyneana was collected from the Masai Plateau, Kolhapur (74.088507 °E and 16.823377 °N), situated in Western Ghats of India. The plant material was identified using the flora of Kolhapur districts (Sardesai and Yadav, 2002) and deposited in the herbarium of 'The New College Kolhapur' (Maharashtra) under the Accession number: SBF & SSK-01. The shade-dried leaves were finely powdered using a mixer grinder. The 20 g fine powder was added to 100 mL of 99.9% methanol (AR grade) and sonicated for 30 min. The mixture was further kept for extraction on a rotary shaker for 24 h. The extract was centrifuged at 5000 rpm for 10 min. The supernatant was filtered through Whatman filter paper No. 1. The filtrate was stored at 4°C for further analysis and used for GC-MS analysis. The fine powder of leaves was used for SEM-EDX analysis.

GC-MS analysis

GC-MS analysis was performed on an Agilent GC 7890 using an MS 5977 B MSD equipped with an Elite One-Fused HP 5 ms capillary column (30 m, 0.25 mm, 0.25 μ m). Nitrogen was used as the carrier gas and the injection volume was 1 mL with constant flow rate of 20 mL min⁻¹ (split ratio, 20:1). For GC-MS analysis, the injector temperature was kept at 200°C. The initial oven temperature was 50°C withheld for 1 min, and then raised to 250°C at the rate 5°C min⁻¹ with each increment withheld for 1 min and then to 280°C at a rate of 5°C min⁻¹ with each increment withheld for 9 min. The MS data from 40-900 amu were recorded at 70 eV with a mass range of a m/z. A single quadrupole mass spectrometer detector at 150°C was used for MS analysis. The scanning frequency was maintained at 1.7 scans min⁻¹. The elements were identified using the EI source spectral library NIST 2017 (Mass Hunter GC/MS Acquisition B.07.06.2704 18-Jul 2017 Copyright © Agilent Technologies, Inc. Ver. 6.00.34). The molecular formula and molecular weight was recorded from database of National Library of Medicine (<https://pubchem.ncbi.nlm.nih.gov/>).

Sample preparation for scanning electron microscope

The 2 mg fine powder of the samples was placed on adhesive tape and coated with a 5 nm layer of gold. This specimen was scanned using a wide angle X-ray beams at acceleration voltage 20.00 kV

and the microphotographs were captured at magnification x 500 using SEM Jeol model JSM-IT200 from Japan provided by the Rayat Shikshan Sanstha's Instrumentation Department Satara.

RESULTS AND DISCUSSION

GC-MS analysis

GC-MS analysis of methanolic extract of *Anisomeles heyneana* leaves showed the presence of 22 compounds (Table 1; Fig 1). Table 1 shows the retention time, peak area, percentage, and chemical nature of the identified compounds. The major phytochemical compounds identified were dimethyl ether, acetic acid methyl ester, 3,5-dithiahexanol 5,5-dioxide, benzene, 1,4-dichloro, naphthalene decahydro-1-methoxy-, neophytadiene, hexadecanoic acid methyl ester, n-hexadecanoic acid, phytol, 1,2-cyclohexanedimethanol, palmitic acid TMS derivative, hexadecanoic acid 2-hydroxy-1-(hydroxy-methyl) ethyl ester, α -tocospiro A, dl- α -tocopherol, γ -sitosterol, and 13,27-cycloursan-3-one. γ -sitosterol was found in higher concentrations in the leaves, and the compound reportedly significantly contributes to the toxicity leading to DNA damage in human peripheral blood mononuclear cells and also suppresses the growth of cancer cell (Yahya *et al.*, 2021). Wang *et al.* (2023) reported similar observations in colon, breast, pancreatic, stomach cervical, prostate and liver cancer cells. Phytol is a diterpenes alcohol used in vitamin E and vitamin K imitative formulas and employed in production of

Table 1: The compounds identified through GC MS analysis in the methanolic extract of *Anisomeles heyneana* leaves

S. No.	Name of the compound	Retention time (min)	Area (%)	Molecular formula	Molecular weight (g moles ⁻¹)
1	Dimethyl ether	1.326	3.22	C ₂ H ₆ O	46.07
2	Acetic acid, methyl ester	1.364	9.61	C ₃ H ₆ O ₂	74.08
3	Ethane, 1,1-dimethoxy	1.565	1.15	C ₄ H ₈ O ₂	90.12
4	Trichloromethane	1.660	0.45	C ₂ H ₅ O ₂	119.38
5	Silane, dimethoxydimethyl-	1.823	0.82	C ₃ H ₆ Cl ₃	120.22
6	Cyclohexane	1.880	0.50	C ₆ H ₁₂	84.16
7	3,5-Dithiahexanol 5,5-dioxide	1.976	3.95	C ₄ H ₁₀ O ₃ S ₂	130.22
8	2,4-Dimethyl-1-heptene	4.013	0.49	C ₉ H ₁₈	126.23
9	Benzene, 1,4-dichloro	8.641	22.74	C ₆ H ₄ Cl ₂	147.002
10	2,4,6-Trimethyl-1-nonene	9.970	0.22	C ₁₂ H ₂₄	168.31
11	2,7-Octanedione	16.539	0.73	C ₈ H ₁₄ O ₂	298.34
12	Naphthalene, decahydro-1-methoxy	24.638	5.12	C ₁₀ H ₁₈	158.19
13	Neophytadiene	28.817	3.30	C ₂₀ H ₃₈	278.51
14	Hexadecanoic acid, methyl ester	30.605	7.78	C ₁₇ H ₃₄ O	270.45
15	n-Hexadecanoic acid	31.676	34.08	C ₁₆ H ₃₂ O	256.40
16	Phytol	34.162	4.66	C ₂₀ H ₄₀ O	296.53
17	1,2-Cyclohexanedimethanol	35.261	13.29	C ₈ H ₁₆ O ₂	144.21
18	Palmitic acid, TMS derivative	40.826	6.51	C ₁₉ H ₄₀ O ₂ Si	328.60
19	α -Tocospiro A	46.296	14.91	C ₂₉ H ₅₀ O ₄	462.70
20	dl- α -Tocopherol	50.685	9.37	C ₂₉ H ₅₀ O ₂	416.68
21	γ -Sitosterol	54.577	82.04	C ₂₉ H ₅₂ O ₂	414.70
22	13,27-Cycloursan-3-one	56.040	100.0	C ₃₀ H ₄₈ O	424.70

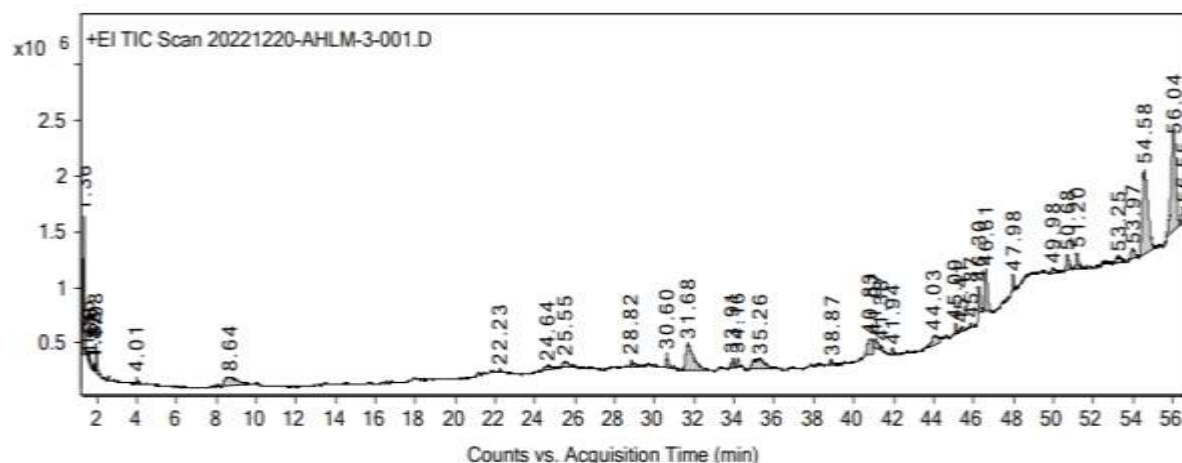


Fig. 1: Chromatogram of GCMS analysis of methanolic extract of *A. heyneana* leaves

cosmetics, shampoo, bathroom soaps, and home cleaning products (Netscher, 2007; McGinty *et al.*, 2010). It also possesses antiradical, antimicrobial, anti-schistosomiasis, anti-inflammatory, antianxiety and antidiabetic properties (Taj *et al.*, 2021). Hexa-decanoic acid reportedly shows antifungal, antimicrobial, and antioxidant activities and decreases blood cholesterol (Muflihunna *et al.*, 2021). The n-hexadecanoic acid is a fatty acid that exhibits anti-atherosclerotic, antimutagenic, antimicrobial, anti-oxidant, antihypoxic, sclerosing, aesthesis and anti-seborrheic activity (Hrichi *et al.*, 2022; Nabi *et al.*, 2022). In present study neophytadiene was also observed in leaves and it is a class of diterpenoids employed in antimicrobial, antifungal, antioxidant and anti-inflammatory activity (Kumari *et al.*, 2022). 1H-indene, 1-hexadecyl-2, 3-dihydro exhibited antiangiogenic and anticancer activity (Xu *et al.*, 2023). Cyclohexane is an alicyclic hydrocarbon which possesses many biological activities anti-inflammatory, antioxidant, antimicrobial and cytotoxic effects (Shoaib, 2019).

The SEM images (Fig. 2) revealed a flake like morphology in the powered extract. Several different chemical compounds were found in *A. heyneana* leaf extract. Therefore, energy dispersive spectroscopy analysis showed the relative abundance of the elements. Carbon, oxygen, magnesium, chlorine, potassium calcium, magnesium, iron, zinc, aluminium, silicon, and phosphorus were present in the sample (Table 2). This study showed found that carbon had the highest weight concentration ($48.76 \pm 0.34\%$) amongst different elements in the extract followed by oxygen ($43.41 \pm 0.68\%$), The other element noticed were calcium ($2.47 \pm 0.11\%$), potassium ($1.86 \pm 0.09\%$), chlorine ($1.31 \pm 0.07\%$),

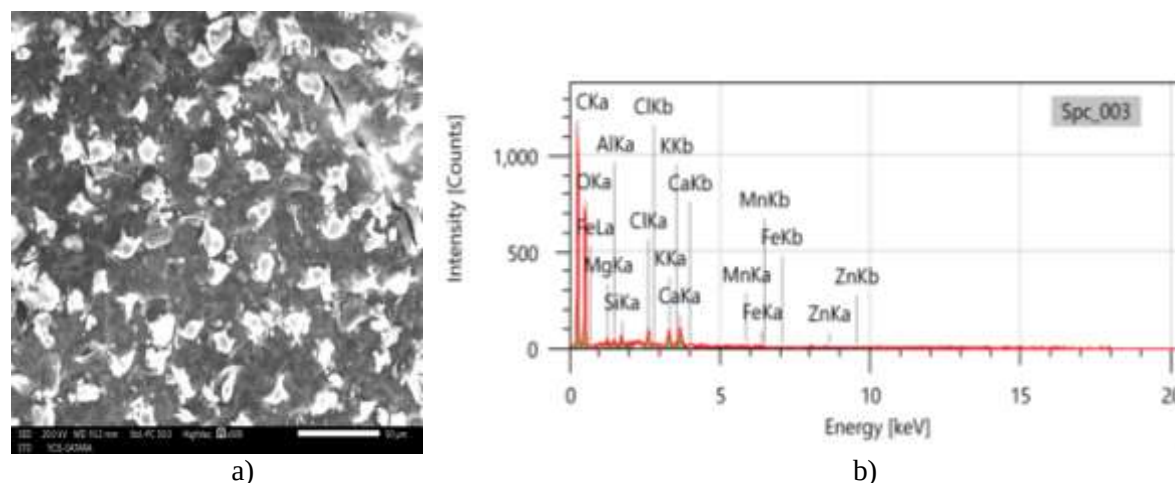


Fig. 2: Elemental analysis of *Anisomeles heyneana* leaf; a) shows the area from where energy dispersive X-ray has been taken, and b) is the energy dispersive X-ray spectroscopic peak of the sample

Table 2: The percentage of elements identified through EDX in *A. heyneana* leaves

Element	Mass (%)	Atom %
Carbon (C)	48.76 ± 0.34	58.10 ± 0.40
Oxygen (O)	43.41 ± 0.68	38.83 ± 0.61
Magnesium (Mg)	0.47 ± 0.05	0.28 ± 0.03
Aluminium (Al)	0.31 ± 0.04	0.17 ± 0.02
Silicon (Si)	0.63 ± 0.05	0.32 ± 0.003
Phosphorus (P)	0.08 ± 0.03	0.04 ± 0.01
Chlorine (Cl)	1.31 ± 0.07	0.53 ± 0.03
Potassium (K)	1.86 ± 0.09	0.68 ± 0.03
Calcium (Ca)	2.47 ± 0.11	0.88 ± 0.04
Manganese (Mn)	0.19 ± 0.05	0.05 ± 0.01
Iron (Fe)	0.31 ± 0.06	0.08 ± 0.02
Zinc (Zn)	0.21 ± 0.10	0.05 ± 0.02
Total	100	100

silicon ($0.63 \pm 0.05\%$), magnesium ($0.47 \pm 0.05\%$), aluminium ($0.31 \pm 0.04\%$), iron ($0.31 \pm 0.06\%$), zinc ($0.21 \pm 0.10\%$), manganese ($0.19 \pm 0.05\%$) and phosphorus ($0.08 \pm 0.03\%$). Oxygen is essential for several cellular biochemical and physiological process while magnesium accounts for 40% of muscle and 1% of fluid in the human body (Zinicovscaia *et al.*, 2020). Calcium is the primary component of bones and teeth, it functions on cell membranes, and regulates blood pressure (Ciosek *et al.*, 2021) whereas chlorine is essential for normal neurophysiological, digestive and renal function (Lamari *et al.*, 2016). Hemoglobin, myoglobin and a plethora of

nutrients rely on iron for their structure and function (Narayanam *et al.*, 2021). Numerous studies have been demonstrated a relation between foods and the occurrence cardiovascular diseases. Copper is necessary for iron metabolism because it is a component of many enzymes (Karaaslan, 2017). Sodium (Na) is essential for maintaining proper cellular ionic balance and bodily system regulation (Bernal *et al.*, 2023). Both red blood cell development and thyroid stimulation requires cobalt (Begaa and Messaoudi, 2018). The beneficial effect of zinc on normal growth, fertility, cell proliferation, tissue differentiation, DNA synthesis, photochemical process of vision, ossification, wound healing and disease treatment are all widely known (Fallah *et al.*, 2018). Presence of all these elements in leaves of *A. heyneana* its nutritive value.

Conclusion: In current study, the GC-MS analysis has shown the presence of 22 bioactive compounds in the methanolic extract of *Anisomeles heyneana* leaves that were identified to exhibit pharmaceutical relevance. Various elements in powder extracts are crucial for secondary metabolites production and exhibits potential impact on human health. The compounds in *A. heyneana* exhibit various properties antidiabetic, antioxidant, antiinflammatory, antibacterial and antifungal activities which indicated its potential for medical application and notable nutritional value.

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